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SUDDEN APPARENTLY UNEXPLAINED DEATH DURING INFANCY

II. PATHOLOGIC FINDINGS IN INFANTS OBSERVED TO DIE SUDDENLY *

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It has been shown that histologic study of infants found dead while in apparent good health and in whom gross necropsy findings were inconclusive, discloses significant disease, mainly of the respiratory tract.[1] In the past such infants frequently were certified as having died of accidental mechanical suffocation, partly because in many of these cases the circumstances of death suggested the possibility of smothering, and partly because of failure to perform complete necropsies or to make proper evaluation of the pathologic findings.

In this paper we are presenting the pathologic findings in infants 12 months of age or under, whose transition from apparent health to sudden death in a matter of minutes was observed by a responsible adult. They expired after a few brief symptoms such as convulsions, cyanosis, or gasping respirations. In the 26 such cases that we encountered from 1931 to 1951 in the Borough of Queens, New York City, mechanical suffocation is excluded with certainty. In 10 cases the gross necropsy findings were sufficient to explain death (Table I).

It is apparent that in all but one case heart disease was present, and in that one case interstitial bronchopneumonia was found. Microscopic evidence of respiratory disease was present in 7 of the 9 cases with developmental anomalies of the heart.

Our main concern in this report is with the 16 cases in which gross

* The third paper of the series, entitled "Pathologic Findings in Infants Dying Immediately after Violence, Contrasted with Those after Sudden Apparently Unexplained Death," is the succeeding article in this issue.

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necropsy findings were inconclusive. These are the cases in which death remained unexplained until microscopic studies were completed. These 16 cases are summarized in Table II.

In 6 of the 16 infants all symptoms of previous illness were denied. Five of the remaining 10 infants had a "cold" at the time of death.

TABLE I

Summary of Findings in Infants Observed to Die While in Apparent Health with Conventional Gross Cause of Death

Case no.	Sex	Age	Prior symptoms	Gross necropsy findings
A 3788	F	2 wks.	One attack of cyanosis in a.m.; found limp but still breathing, and died	Congenital heart disease
A 3252	M	2 wks.	Breathed heavily at times; refused bottle, had a peculiar stare and died	Glycogen tumor of heart
A 3174	F	10 mos.	Always well; found with eyes rolled back but still breathing; expired	Cardiac hypertrophy (idiopathic); broncho-pneumonia; mastoiditis
M 432	M	8 mos.	Always well; suddenly screamed, kicked about, and died	Congenital heart disease
ME 40-38	M	6 mos.	Seemed to breathe heavily for a few days; found "shaking" and expired	Congenital heart disease
ME 43-295	M	2 mos.	Previously well; found limp and gasping; then died	Idiopathic cardiac hypertrophy
ME 44-297	F	11 days	Nursed poorly for 2 days; became "livid" day before death for a short period; at 3:15 a.m. suddenly expired	Congenital heart disease
ME 45-9	F	6 wks.	Had a "cold" for 9 days; had one convulsion 3 days before, then appeared to be well, suddenly screamed and expired	Interstitial broncho-pneumonia; bilateral hydrothorax
ME 46-81	F	3 wks.	Expired suddenly in mother's arms	Congenital heart disease
ME 48-92	F	1 mo.	"Feeding problem"; otherwise apparently well; suddenly gasped and died	Congenital heart disease

Of the other 5, one had respiratory difficulty described as "difficult breathing," one was a "feeding problem," one "did not look well" the morning before death, one had "turned stiff" and cold for a minute the day before death, and one had loose bowel movements the day before.

Because of the rapidity of death it was truly fortuitous that these infants were observed to die and not found dead. Infants are often

left alone for hours, particularly when they are believed to be asleep. Thus the extreme rarity with which these cases are encountered in medicolegal practice can be understood.

Representative Cases

Case 6 (ME 42–235)

According to the mother, this 4-months-old white male had a cold for 1½ weeks. Fifteen minutes after she had put him to sleep he was found "white" and drenched with perspiration. Respirations were labored and in a few minutes ceased.

At necropsy the lungs were voluminous and congested. There was an indefinite nodular sensation on palpation, but on section no consolidation was visible grossly. There was no aspiration of stomach contents. The mastoids were normal.

Microscopic Findings. In sections of the lungs the alveoli in large areas were filled with alveolar macrophages, edema fluid, numerous lymphocytes, many of which were pyknotic, and a moderate number of polymorphonuclear cells. There was lymphocytic infiltration of the alveolar walls. Intra-alveolar erythrocytophagocytosis was conspicuous. The bronchial walls showed edema and in some there was cellular infiltration of monocytes and occasional polymorphonuclear cells. The bronchial epithelium showed degeneration and in some of the lumina there were a few polymorphonuclear leukocytes and lymphocytes. There was submucosal edema of the pharynx and infiltration by lymphocytes, many of which were pyknotic. Similar change was seen in the larynx and trachea. The lymphatics were dilated. Edema of the epiglottis was conspicuous. In the cervical lymph nodes erythrophagocytosis and pyknosis of lymphocytes was prominent. There were perivascular hemorrhages in the brain. In the thymus abundant nuclear and cytoplasmic débris was found in Hassall's corpuscles.

Post-mortem cultures yielded a mixed flora of non-pathogenic organisms.

Case 8 (ME 44–308)

According to the mother, this 8-months-old child had a cold without cough for 2 weeks. She suddenly screamed, began to vomit, lost consciousness, and died.

The only gross abnormality at necropsy was massive aspiration of stomach contents into the tracheobronchial tree. The mastoids were normal.

Microscopic Findings. Scattered through sections of the lungs were a moderate number of foci of intra-alveolar and interstitial lymphocytic infiltration. Many of these cells were pyknotic (Fig. 2). Within the alveolar lumina there were also alveolar macrophages, among them

TABLE II

Summary of Findings in Infants Observed to Die While in

Case	Age and sex	Recent history	Symptoms immediately before death	Gross findings
No. 1 ME 36-33	4 mos. Female	Apparently well	"Fainted" in mother's arms and died	Lungs: congestion and edema; mastoids: not described
No. 2 ME 40-46	1 mo. Male	Apparently well	Found in convulsions and died	Lungs: 140 gm., congestion and edema; purulent mastoiditis
No. 3 ME 40-48	7 wks. Female	Apparently well	Found gasping and died	Lungs: 140 gm., edema and congestion; mucoid material in smaller bronchi; mastoids: not described
No. 4 ME 42-61	5 wks. Male	Turned "stiff and cold" for a minute the day before	Stopped breathing, became cold and clammy, and died	Lungs: edema and congestion; mastoids: not described
No. 5 ME 42-98	6 wks. Female	Stated to be "feeding problem" (well nourished)	Vomited and died	Lungs: 108 gm., edema and congestion; trachea and bronchi congested; small amount of aspirated stomach contents in trachea; mastoids: not described
No. 6 ME 42-235	4 mos. Male	"Cold" 1½ weeks	Found drenched with perspiration, white, breathing heavily, and died	Lungs: congestion and edema; mastoids: normal
No. 7 ME 44-273	1 mo. Male	"Cold" 2 days	Found "sick"; died while awaiting physician	Lungs: 110 gm., congestion and edema; trachea and bronchi congested; smaller bronchi contain mucoid material; mastoids: normal; diaper dermatitis
No. 8 ME 44-308	8 mos. Male	"Cold" 2 weeks	Screamed, vomited, became limp, and expired	Lungs: 140 gm.; trachea and bronchi filled with aspirated stomach contents; mastoids: normal
No. 9 ME 44-330	3 mos. Male	None	Found breathing heavily and died	Lungs: 120 gm.; edema and congestion; mucoid material in smaller bronchi; aspiration of stomach contents; Mastoid: normal
No. 10 ME 46-80	2 mos. Female	Slight "cold" 1 week ago	"Shook, became red," and died while in mother's arms	Lungs: congestion and edema; purulent mastoiditis
No. 11 ME 47-71	1 mo. Female	Two episodes of "difficult" breathing; declared normal by examining physician 1 week before	Died while being fed	Lungs: 200 cc. of clear fluid in each pleural cavity; congestion and edema; trachea and bronchi congested; mastoids: contained serous fluid
No. 12 ME 47-125	3 wks. Female	Several loose bowel movements day before	After feeding, rolled eyes, turned white, and died	Lungs: aspiration of stomach contents, patchy collapse; mastoid: normal
No. 13 ME 51-92	10 days Male	Did not "look well" morning of death	Found breathing heavily and died	Lungs: 70 gm., congestion and edema; aspiration of stomach contents; serous fluid in mastoids
No. 14 A 1897	2 mos. Female	"Cold" 1 week	"Cranky," became blue and cold on way from doctor's office, and died	Lungs: edema and congestion; mastoids: not described
No. 15 A 3308	7 wks. Male	None	Found gasping, lying on back, and died	Lungs: congestion and edema; mastoids: normal
No. 16 A 3876	6 days Male	None	Sudden difficulty in breathing, expired in 15 minutes	Lungs: congestion and edema; mastoids: normal

Apparent Health, with Insufficient Gross Findings to Explain Death

Microscopic findings			Bacteriologic findings
Upper respiratory tract	Lung	Other significant findings	
Tracheitis	Bronchitis, congestion, focal interstitial pneumonitis	Intestine and pancreas: interstitial edema	
Laryngitis	Bronchitis, collapse and congestion, focal interstitial pneumonitis	Focal mononuclear infiltration of endocardium; focal adrenal hemorrhage; waxy degeneration of muscle in tongue	No pathogens
Acute suppurative tonsillitis, acute laryngitis, tracheitis	Bronchitis, collapse, congestion, and hemorrhage	Thymus: interstitial edema; pulmonary thrombi	Lungs: pneumococcus
Laryngotracheitis	Bronchitis, congestion, and hemorrhage	Thymus: interstitial edema; focal glomerulonephritis; focal adrenal hemorrhage	Spleen, lungs, trachea, and bronchi: *Streptococcus haemolyticus*
No sections	Bronchitis, collapse, congestion, hemorrhage, and interstitial edema	Mesenteric arteriosclerosis; focal adrenal hemorrhage	Heart's blood: *Strep. haemolyticus*
Pharyngitis, laryngitis, and tracheitis	Interstitial bronchopneumonia	Thymus: interstitial edema and focal mononuclear infiltration	No pathogens
Tracheitis	Bronchitis, congestion, and hemorrhage	Focal cerebral and adrenal hemorrhage; testis: edema and focal mononuclear infiltration	No pathogens
Acute laryngitis with necrosis; acute suppurative tonsillitis	Interstitial pneumonitis, congestion; bronchitis	Pulmonary thrombi	Lung: sterile
Early pharyngitis and tracheitis; acute suppurative tonsillitis; early mastoiditis	Bronchitis; marked collapse, congestion and edema	Focal perivascular cerebral and adrenal hemorrhages; waxy degeneration of laryngeal muscle	
Pharyngitis, laryngitis, and acute suppurative tonsillitis	Edema and collapse	Pulmonary thrombosis; interstitial edema of pancreas; focal adrenal hemorrhage	Right mastoid: *Staphylococcus haemolyticus*; tonsil, heart's blood: *Strep. haemolyticus*
Laryngitis; early mastoiditis	Interstitial pneumonitis	Thymus and pancreas: interstitial edema	Lung and spleen: sterile
Mastoid: purulent inflammation	Patchy collapse, bronchitis	Liver: fatty change; acute enteritis	No pathogens
Pharyngitis, acute laryngitis with necrosis; acute suppurative tonsillitis	Focal bronchopneumonia, marked collapse, congestion, and hemorrhage	Acute suppurative inflammation of submaxillary gland; thrombi in kidney and lung; focal adrenal hemorrhage	No pathogens cultured; numerous bacteria in sections of lung, larynx, and trachea
Acute laryngitis; acute suppurative tonsillitis	Bronchitis, collapse, and congestion	Interstitial nephritis; acute enteritis; focal adrenal hemorrhage	No pathogens
Tracheitis; acute suppurative laryngitis	Bronchitis, congestion, collapse, and interstitial edema	Testis: focal mononuclear infiltration; pulmonary thrombi; kidney: focal glomerulitis and interstitial infiltration	
	Hemorrhage, congestion, and collapse	Focal cerebral and adrenal hemorrhages; pulmonary thrombi	

numerous giant forms. In one section there was diffuse lymphocytic infiltration of the wall of a large bronchus and there was bronchial epithelial degeneration. "Platelet" thrombi were encountered in some pulmonary vessels. The aspirated material noted grossly was usually in the larger bronchi, only occasionally extending into the smallest branches. In the trachea and larynx there was submucosal edema and infiltration by mononuclear cells. Deep within the larynx and just outside the laryngeal cartilage there was a focus of necrosis within the muscle with infiltration by pyknotic lymphocytes. There was meningeal edema and numerous focal subarachnoid hemorrhages with slight mononuclear infiltration.

Culture of the lung was sterile.

Case 10 (ME 46–80)

This infant of 2 months had a slight cold 1 week before death. She was fully dressed and was being taken out of the home in her mother's arms when she suddenly rolled her eyes and died.

Grossly, bilateral purulent mastoiditis was found at necropsy. The lungs showed congestion and edema.

Microscopic Findings. In the interstitial tissues of the lungs there were extensive edema, slight hemorrhage, and infiltration by mononuclear cells, mainly lymphocytes (Figs. 10). A moderate number of alveolar macrophages were seen in the alveolar lumina. Erythrocytophagocytosis was conspicuous. There was no evidence of bronchitis in the sections studied. In the bronchopulmonary lymph nodes there were focal hemorrhages. The hilar lymphatics were dilated and frequently contained blood. Many of the lymphocytes were pyknotic. Several pulmonary vessels contained thrombi (Fig. 11). There was edema of the pharyngeal and laryngeal submucosa and muscle; interstitial edema was conspicuous also in the pancreas. There was focal hemorrhage in the adrenal glands. Mononuclear infiltration in the meninges was conspicuous.

Streptococcus haemolyticus was recovered from the right mastoid, tonsil, and heart's blood.

Case 12 (ME 47–125)

According to the foster-mother, this 3-weeks-old female infant had several loose bowel movements the day before, but had behaved normally the day of death. After a feeding she rolled her eyes, turned white, and died.

At necropsy there was patchy collapse of the lungs and a small amount of aspirated stomach contents in the trachea.

Microscopic Findings. In the lung there was congestion and patchy collapse; macrophages were seen in the alveoli. The bronchi contained aspirated stomach contents. In an occasional bronchus there were mural edema and sparse infiltration by a few polymorphonuclear leukocytes and lymphocytes. There was bronchial epithelial degeneration and desquamation. A fibrin thrombus was seen in a pulmonary vessel. The lining epithelium of the mastoid air cells was edematous and infiltrated by polymorphonuclear leukocytes and mononuclear cells (Figs. 5 and 6). There was polymorphonuclear leukocytic infiltration of the intestinal mucosa. In the submucosa and muscle, mononuclear cells predominated. Many of these cells had acidophilic cytoplasm, and pyknotic lymphocytes were prominent. In the liver there was fatty change, similar to that seen in a child found dead, as illustrated in Figure 35, Part I of this series.[1] In the meninges there were focal hemorrhages and edema.

Cultures of organs, including intestines, revealed no pathogenic organisms.

Case 13 (ME 51–92)

This infant of 10 days was found in collapse, breathing heavily, and died within a few minutes. He had been sent home from the hospital of birth apparently well 3 days before. The grandmother had thought he did not look well the morning of death, but he seemed to behave normally.

At necropsy there was massive aspiration of stomach contents and slight congestion of the tracheobronchial mucosa. The mastoid antra and middle ears were filled with clear fluid.

Microscopic Findings. In the lungs, congestion, and subpleural and interstitial edema were prominent. Several small foci of intra-alveolar exudate consisted mainly of lymphocytes and a few polymorphonuclear leukocytes. The bronchial epithelium was degenerated, the walls edematous, and the lumina contained a few polymorphonuclear leukocytes and lymphocytes. Numerous squames from aspirated vernix were present also in the alveoli. The aspirated material noted grossly was found throughout the tracheobronchial tree. Thrombi were seen in several pulmonary vessels. The pharyngeal wall showed marked edema with cellular infiltration by both polymorphonuclear leukocytes and mononuclear cells. In the true vocal cord there was a large area of necrosis with ulceration of the epithelium and polymorphonuclear leukocytic infiltration (Fig. 4). There was purulent exudate in the ducts of the submaxillary gland with polymorphonuclear cells infiltrating the duct epithelium and occasionally extending into the wall. The cervical lymph nodes were edematous and contained many pyknotic lymphocytes and some polymorphonuclear leukocytes. The tonsillar epithe-

lium was diffusely infiltrated by pyknotic lymphocytes, and the follicles showed edema and increased phagocytosis. The subtonsillar lymphatics were dilated and filled with lymphocytes. In the mastoid there was infiltration of the lining epithelium by acidophilic mononuclear cells. In the meninges there were focal hemorrhages, edema, and slight infiltration by acidophilic mononuclear cells (Fig. 7). In the kidney, in addition to the extreme hyperemia, several thrombi were found (Fig. 8).

Bacteria were numerous in the sections of lungs, larynx, and trachea, although no pathogenic organism was recovered from lung or spleen, and heart's blood and spinal fluid were sterile.

Case 15 (A 3308)

According to the parents, this 7-weeks-old white male infant was entirely well. Forty-five minutes after he had been put in his crib he was found lying on his back, gasping. He was pronounced dead on arrival at a hospital.

The only gross finding at necropsy was congestion of the lungs. The mastoids were normal. There was no aspirated material in the airway.

Microscopic Findings. In the lung there were marked congestion, interstitial edema, and hemorrhage (Fig. 1). The bronchial walls were edematous and infiltrated by mononuclear cells. There were thrombi in several pulmonary vessels. In the epiglottis there was marked interstitial edema with diffuse infiltration of epithelium and submucosal tissues by mononuclear cells. Mononuclear and polymorphonuclear infiltration was conspicuous in the larynx. In the trachea there was very intense mononuclear infiltration extending into the muscle layers. There was conspicuous focal hemorrhage in the adrenal glands. The meninges were edematous and infiltrated by mononuclear cells. There was an occasional perivascular hemorrhage in the brain. There was no abnormality in the one section of mastoid studied.

Microscopic Analysis and Discussion

There was inflammation of the respiratory tract in each of the 16 cases. Acute suppurative tonsillitis was present in 4 of the 7 cases in which tonsillar sections were available. In all tonsils studied there were numerous macrophages in the follicular centers and many pyknotic lymphocytes were seen penetrating the epithelium. In 8 of the 11 cases in which sections of the trachea were taken there was submucosal and epithelial infiltration by mononuclear cells. In one of these, polymorphonuclear leukocytes also were present. In 4 of the 11 cases in which the gross appearance of the mastoid was recorded, 2 had purulent exudation, 2 contained serous fluid. Microscopic study

of the latter 2 showed purulent inflammation. Of the 7 described as grossly normal, sections were available in 4. Two of these 4 showed mastoiditis.

It should be noted that in our most recent series of infants found dead,[1] 90 per cent had gross or microscopic evidence of mastoiditis. This lesion frequently was overlooked in our earlier cases because of failure to examine the mastoids in some cases, to recognize that even a small amount of fluid is abnormal, and to make adequate microscopic study of those cases described as normal at necropsy.

In 14 of the 16 cases in which sections of larynx were available, necrotic laryngitis was found twice; purulent laryngitis without necrosis, twice; 6 had non-suppurative inflammation; in 4 there was no significant change. We have found that the necrotic lesion may be limited to a very small area of the vocal cord, and may be easily missed unless multiple sections are taken.

In 11 of the 16 cases bronchitis was present. This was non-suppurative in all cases except in the 2 in which there was bronchopneumonia. One of these 2 infants also had interstitial pneumonia. Interstitial pneumonia alone was found in 3 cases (Figs. 2 and 3). Congestion, edema, hemorrhage, and collapse were present in most of the lungs (Figs. 1 and 9). It seems reasonable to suggest that both in these infants and in those *found dead* the incidence of bronchopneumonia as it is ordinarily seen might have been encountered more frequently if the infants had survived longer. Pulmonary congestion is classically described as the first stage in pneumonia.

Aspirated material was seen grossly in 4 cases and microscopically in one additional case. In Part III of this series we will show that aspiration may occur as an agonal phenomenon in violent deaths. In our experience, aspiration has been a cause of death only under special circumstances such as the presence of a foreign body in the airway or an abnormal restraining position as a result of which vomitus was inhaled.[2] In those infants observed to die who showed aspiration, there were the same pathologic changes as in those infants without aspiration. This was true also of the infants found dead. ME 44–309, illustrated in Figures 5 and 6 of Part I of this series,[1] an infant *found dead* the same day as Case 8 (ME 44–308) (Fig. 2) was *observed to die,* had identical changes without aspiration. It may be of some interest that these two deaths occurred in the same vicinity. It is reasonable to assume that the state of collapse which preceded death also preceded the aspiration.

Thrombosis was seen in 6 of the 16 cases in the sections examined.

In case 13 (ME 51–92) thrombi were most numerous and were present in both pulmonary and renal vessels (Fig. 8). In 5 they were found in lung or brain and other regions (Fig. 11). Focal adrenal hemorrhages were seen in 7 of the 16 cases. Hyperemia and hemorrhage of bronchopulmonary and cervical lymph nodes were common findings.

Interstitial edema was present in many visceral sections in addition to the lung, particularly the meninges (Fig. 7), thymus, and pancreas.

Focal glomerulitis was seen in 6 of the 16 cases and was advanced in one case. In 2 instances, in addition, there was interstitial cellular infiltration. Interstitial cellular infiltration was found in the liver in 5 cases, in 2 of which it was fairly extensive. Focal mononuclear cellular infiltrate also was seen twice in the testis, once in the endocardium, once in the submaxillary gland, and once in the thymus. The presence of enteritis in 2 cases, one of which had fatty change of the liver, shows that in these instances there was associated involvement of the alimentary tract. In one of these cases there was mastoiditis; and in the other, bronchitis and suppurative laryngitis. The association of diarrhea with mastoiditis is well known. It is not so well known, however, that mastoiditis in infancy may be associated with syncope, collapse, or sudden death.[2]

The infants in this series died in collapse or in convulsions. It has been recognized that these are often the first signs of profound infection, especially during infancy. In case 4 (ME 42–61) there was apparent recovery from an episode of collapse 1 day before death. Among the infants found dead, one (ME 49–18) had been hospitalized following collapse and fever approximately 2 weeks before death. She had been discharged apparently completely recovered, although no clinical diagnosis had been established. On the fourth day after discharge she was found dead in her crib, 3 hours after she had been put to sleep. Histologic changes consistent with a diagnosis of respiratory disease were found also in this case.

Summary and Conclusion

For a series of infants *observed to die* suddenly while in apparent good health, in whom the gross necropsy findings were inconclusive, histologic lesions are described consisting of inflammatory changes in the respiratory tract, vascular changes, focal cellular infiltrations in many organs, and reaction of the lymphatic tissues.

These lesions are identical with those we have previously reported in babies *found dead* in whom mechanical suffocation is often alleged

as the cause of death. The present study offers additional evidence for the previous conclusions; namely, that in infants, fulminating respiratory disease is the common cause of sudden, unexpected, and apparently unexplained death.

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[*Illustrations follow*]

LEGENDS FOR FIGURES

FIG. 1. Case 15 (A 3308). Male, 7 weeks old. Observed to die. Lung: Interstitial hemorrhage; extreme congestion. × 34.

FIG. 2. Case 8 (ME 44–308). Male, 8 months old. Observed to die. Lung: Infiltration of alveolar lumina and walls by mononuclear cells. Intra-alveolar edema may be noted also. × 34.

FIG. 3. Case 11. (ME 47–71). Female, 1 month old. Observed to die. Lung: Interstitial pneumonitis. Interstitial edema and infiltration are marked. The alveolar walls have slightly increased cellularity. × 11.

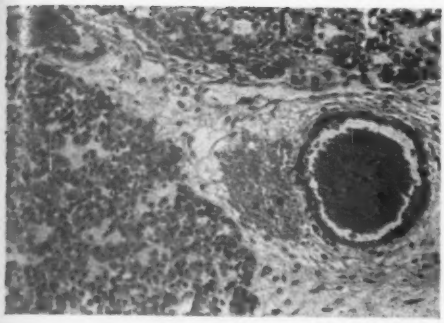
FIG. 4. Case 13 (ME 51–92). Male, 10 days of age. Observed to die. Larynx through vocal cord: Extensive degeneration of epithelium with necrosis, polymorphonuclear leukocytic and lymphocytic infiltration in the connective tissue. × 70.

FIG. 5. Case 12 (ME 47–125). Female, 3 weeks old. Observed to die. Mastoid: Infiltration of epithelium by polymorphonuclear leukocytes and mononuclear cells. This mastoid was grossly normal. × 34.

FIG. 6. High power view of Figure 5. × 146.

FIG. 7. Case 13 (ME 51–92). Male, 10 days of age. Observed to die. Brain: Meningeal edema, hyperemia, and mononuclear infiltration. × 6.

FIG. 8. Case 13 (ME 51–92). Male, 10 days old. Observed to die. Kidney: Hyperemia; thrombus in vessel. × 42.



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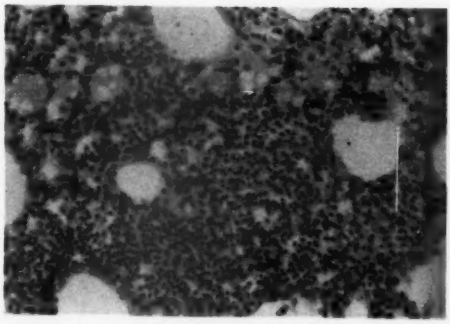

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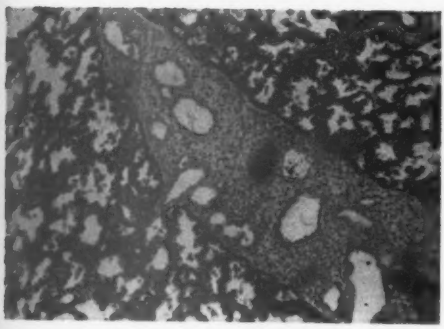

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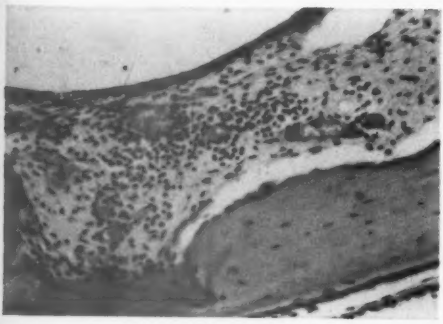

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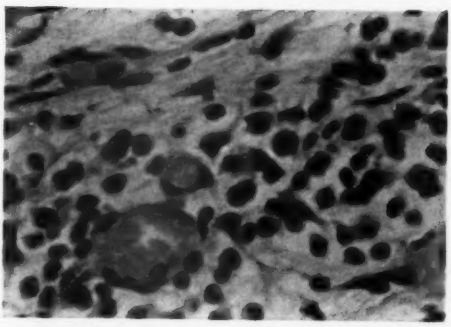

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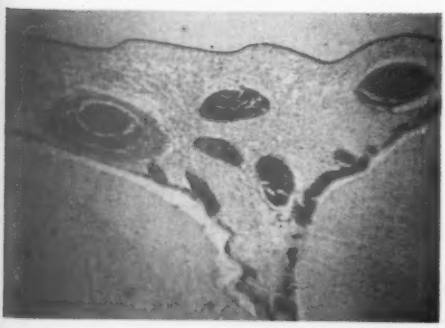

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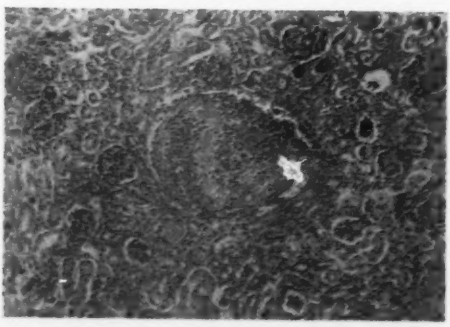

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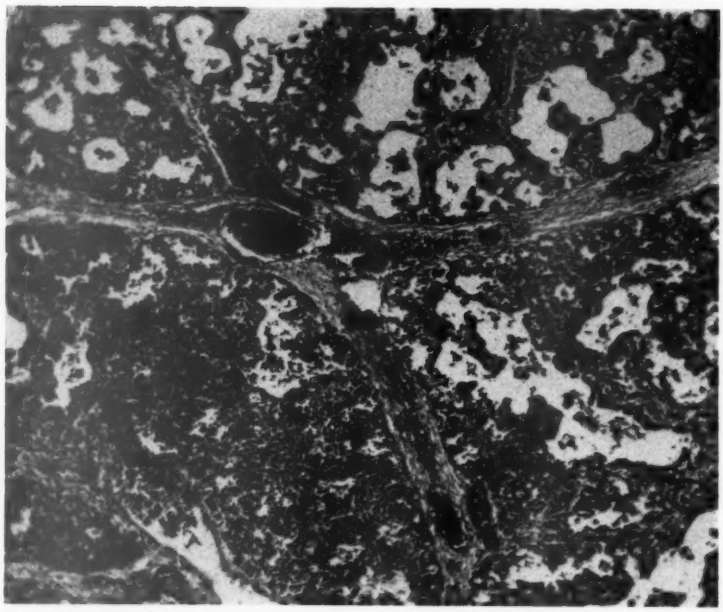
FIG. 9. Case 16 (A 3876). Male, 6 days old. Observed to die. Lung: Collapse, extreme congestion, focal intra-alveolar and interstitial hemorrhage. × 60.

FIG. 10. Case 10 (ME 46–80). Female, 2 months of age. Observed to die. Lung: Interstitial edema and hemorrhage. There are macrophages in some alveoli. There is slight collapse. A subpleural hemorrhage is also visible. × 66.

FIG. 11. Same case as that from which Figure 2 was taken. Lung: Thrombus in vein. Also scattered macrophages in the alveoli. × 110.



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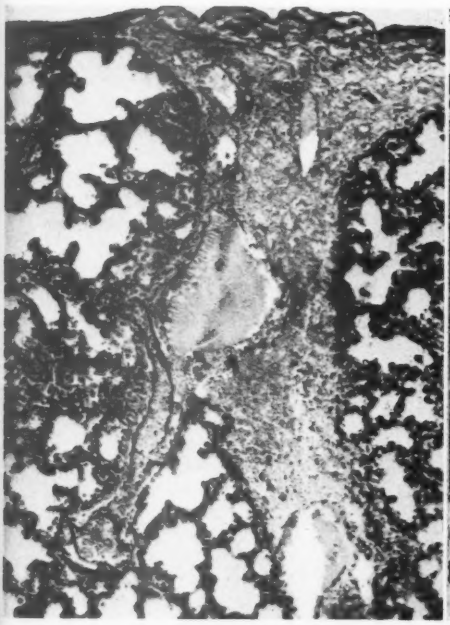

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SUDDEN APPARENTLY UNEXPLAINED DEATH DURING INFANCY

III. Pathologic Findings in Infants Dying Immediately after Violence, Contrasted with Those after Sudden Apparently Unexplained Death *

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In attempting to evaluate the histologic changes in infants who died suddenly while in apparent good health and in whom the gross necropsy findings were insufficient to explain death,[1] we have found it essential to compare the microscopic findings in these cases with those of infants dying rapidly of unquestioned violence. Only by the study of such control material can one establish what is normal for this age group.

In a routine hospital necropsy service such normal viscera rarely are available for study, since violent deaths usually are referred to the medicolegal authorities. Furthermore, violent deaths are not common in infancy. In the few that do occur, the cause of death usually is evident and therefore these cases are seldom necropsied.

In this paper we are reporting upon the pathologic study of 26 consecutive cases in which the infants died immediately after violence.† Of these, 24 were between 17 days and 18 months of age. This period is within the age range of almost all of our cases of sudden apparently unexplained death during early life. The majority of such cases of sudden death occur within the first 6 months of life and are quite rare after the first year.

Also included in this material are 2 cases falling outside the age period with which we are particularly concerned: one newborn dying of manual strangulation, and a 4-year-old child dying of homicidal suffocation by a pillow. They are included because they represent types of asphyxial death not encountered in the other 24 cases. They also serve as control material for the few cases seen in the neonatal period and in early childhood.

The scarcity of normal control tissues from infants under the age of 7 months (dying immediately after trauma), excluding the new-

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† Two of these infants survived burns ½ and 1 hour, respectively.

born, is illustrated by the fact that in a medicolegal experience of 20 years in the Borough of Queens, New York City,* we have been able to accumulate only 10 such cases, as is seen in Table I.

There are no reports in the literature on the weights of organs of infants dying immediately after violence. Because of this lack of information undue significance frequently has been attached to the presence of a "large" thymus gland (status thymicolymphaticus) and "small" adrenal glands (so-called paper thin). Table I shows great variation in the weights of the thymus and adrenal glands of "normal" infants, who were well nourished and free of significant disease. Thymic weights as high as 60 gm. and as low as 18.5 gm. were noted for the same age (Fig. 10). The smallest adrenal glands, weighing 1.6 gm., were found in case 16, an 8-months-old infant dying of illuminating gas poisoning. Because of the variation found in the present control material, the size of thymus and adrenal glands does not seem to be related to the cause of death.

In the infants dying suddenly while in apparent good health, and in whom the gross necropsy findings were inadequate to explain death, we have described two categories of lesions: one local in the respiratory tract and the other comprising vascular, lymphatic, and parenchymal changes. Our observations in this series of "violent deaths" will therefore be presented in these two groups.

Respiratory Tract

Lungs

Since the pulmonary findings were found to vary with the manner of death, they will be discussed in relation to the cause of death.

Drowning. Five infants (cases 2, 5, 12, 13, and 22) drowned. Grossly, the lungs were light pinkish tan and crepitant (Fig. 9 of this paper and Fig. 2 of Part I of this series[1]). From the cut surface a large amount of watery fluid exuded; pleural petechiae were absent or inconspicuous. Microscopically, the bronchi and pulmonary parenchyma in cases 2, 5, and 12 (ages 17 days, 2½ months, and 7 months) were essentially normal (Figs. 1 and 13; also Figs. 4, 26, 27, and 47 of Part I of this series[1]). Occasional areas in the periphery showed patches of slight collapse and intra-alveolar hemorrhage (Fig. 14). Accumulations of alveolar macrophages were seen within these areas (Fig. 21). In case 13 (8 months old) the lung was normal except in one section where there was bronchial mural lymphocytic infiltration

* Population now 1,500,000.

TABLE I
Infants Dying Immediately after Trauma

Case no.	Sex	Age	Cause of death	Organ weights in grams	
				Thymus	Adrenal gland
1. ME 43-19	F	Newborn	Manual strangulation		
2. ME 47-112	M	17 days	Drowning	8.4	4.8
3. A 3075	F	2 mos.	Extensive burns	40.8	3.0
4. ME 49-5	M	2 mos.	Suffocation by live steam	25.9	1.8
5. ME 46-101	F	2½ mos.	Drowning	18.5	2.6
6. ME 43-143	M	3 mos.	Aspiration of stomach contents following impaction of safety pins in pharynx and esophagus	25.0	2.45
7. ME 47-47	F	3 mos.	Illuminating gas poisoning	28.1	2.0
8. ME 47-27	M	3 mos.	Extensive burns	29.0	2.3
9. ME 48-109	F	6 mos.	Suffocation by live steam	22.5	2.3
10. A 2582	M	6 mos.	Death under ether anesthesia for repair of cleft palate	40.0	
11. ME 46-82	M	6 mos.	Extensive burns	33.7	3.6
12. ME 39-122	F	7 mos.	Drowning	40.0	
13. ME 42-65	M	8 mos.	Drowning	18.5	2.0
14. ME 44-44	F	8 mos.	Aspiration of stomach contents following suspension from bed by shoulders in sleeping bag	30.0	1.6
15. ME 48-13	M	8 mos.	Aspiration of stomach contents following wedging of head in angle of crib	46.9	2.5
16. ME 51-62	M	8 mos.	Illuminating gas poisoning	27.0	1.5
17. ME 44-114	M	8 mos.	Strangulation by harness	28.3	2.8
18. ME 42-196	M	8 mos.	Strangulation by rope, homicide	60.0	
19. ME 51-257	M	9 mos.	Suffocation by live steam	50.0	
20. 44-RA-15	F	9 mos.	Extensive burns	17.4	1.9
21. ME 42-67	M	9 mos.	Extensive burns	31.0	3.0
22. ME 45-75	M	9 mos.	Drowning	22.7	3.7
23. ME 50-144	M	16 mos.	Asphyxia by aspiration of lollypop	47.5	3.0
24. 51-A-95	M	18 mos.	Asphyxia by compression of chest by overturned dresser		
25. 42-A-72	F	18 mos.	Asphyxia by blood clot surrounding impacted eggshell in larynx	32.0	
26. A 3809	M	4 yrs.	Homicidal suffocation by pillow	30.6	3.1

and epithelial degeneration. In the lung parenchyma immediately adjacent to this area of bronchitis there was thickening of the alveolar wall, mainly due to the presence of numerous mononuclear cells. This histologic appearance warranted a diagnosis of focal interstitial pneumonitis. In this case serous fluid was found in both mastoids. It is of interest that this infant was described as having been "extremely cranky" the day that it was accidentally drowned.

One other case (no. 22), 9 months old, also had focal interstitial pneumonitis similar to that described in the preceding cases (Fig. 11). An adjacent bronchus and a section of trachea showed non-suppurative mural infiltration. Elsewhere in this infant's lung there was a single nodule, approximately 0.5 cm. in diameter, which in the gross resembled a tuberculous focus. Microscopically, this proved to be an area of organized pneumonia (Fig. 12). This infant had had whooping cough 6 weeks before. It should be emphasized that except for these two small areas, the lung was normal (Fig. 13).

Carbon Monoxide Asphyxia. In cases 7 and 16 death resulted from accidental illuminating gas poisoning. Blood carbon monoxide saturations of 65 and 60 per cent were found. In both cases the blood and viscera showed typical cherry-red discoloration. Pleural petechiae were not seen. Both cases showed gross pulmonary edema. Microscopically, the lungs showed widespread intra-alveolar edema and patchy atelectasis (Fig. 17). Congestion of alveolar capillaries was moderate. The number of intra-alveolar macrophages varied from one section to another. In some areas they were extremely numerous, resulting in complete filling of the alveoli. The bronchi showed only slight epithelial degeneration; there was no mural infiltration, hyperemia, edema, or mucous gland degeneration.

Conflagrations. The infants designated as cases 3, 8, 11, 20, and 21 died in conflagrations. In cases 3 and 21 the infants were burned in an open carriage out-doors and survived ½ and 1 hour, respectively. Carbon monoxide saturation of less than 2 per cent was found. In case 8 the carbon monoxide saturation of the blood was 65 per cent. In these cases the bronchial epithelium showed marked desquamation and degeneration as a direct effect of the extreme exposure to smoke (Fig. 2). The injured epithelial cells were mixed with mucus and carbon particles, frequently occluding the lumina. There was no evidence of mural bronchitis. Alveolar capillary congestion, when present, was slight. There was also slight to moderate patchy collapse and slight intra-alveolar edema; in some cases the alveolar macrophages were numerous (Fig. 17). Interstitial edema or hemorrhage was rarely seen

and when present was of limited extent in comparison with the lungs of infants "found dead."

Suffocation by Steam. Bronchial epithelial degeneration and desquamation were seen also in the 3 infants dying of suffocation and live steam (Fig. 3). At necropsy the denuded epithelium admixed with edema fluid actually poured from the bronchi. The pulmonary parenchymal changes were less marked but similar to those seen in illuminating gas poisoning.

Miscellaneous. Of the remaining 11 cases, 3 infants died of strangulation (Fig. 4), 3 of aspiration of stomach contents (Figs. 15 and 18), 2 of impaction of foreign body in larynx (Figs. 5 and 19), one of homicidal asphyxiation by a pillow, one of asphyxia by compression of chest by an overturned dresser (Fig. 20), and one by overdose of ether during repair of cleft palate. Varying degrees of collapse and of intra-alveolar edema with intra-alveolar accumulations of macrophages and lymphocytes were again encountered. Deviation from the normal was least marked in case 10 (death under ether anesthesia) and in case 25 (asphyxia by blood clot surrounding impacted eggshell). Areas of normal lung, uninvolved by edema or collapse, were seen in all cases in this group, although the amount of normal lung seen varied considerably. Slight mural bronchial lymphocytic infiltration was found in 3 cases (nos. 17, 18, and 24) and marked infiltration in 2 (nos. 23 and 26).

Mastoids

Grossly normal mastoids were reported in 21 cases. In 4 cases the mastoid antrum and middle ear contained serous fluid or mucoid material. In 2 of these 4 cases sections were available and microscopic study confirmed the presence of early inflammation. Of the 10 grossly normal mastoids that were studied microscopically, 7 were normal (Fig. 37 of Part I of this series[1]); 3 had infiltration of the epithelium lining the air spaces by lymphocytes and eosinophils. In 2 of the 3 latter cases there was also infiltration by a few polymorphonuclear leukocytes. Thus, in 7 cases there was evidence of early mastoiditis, although frank pus was never seen grossly.

Tonsils

In 14 cases sections of the tonsil were available for study. All but 2 contained follicles with very distinct borders (Fig. 45 of Part I of this series[1]). Even in the 3 cases that showed a few polymorphonuclear leukocytes in the crypts, the follicular borders were distinct.

Larynx and Trachea

In 4 cases there was moderate, and in 3 cases there was slight diffuse lymphocytic infiltration of the submucosa of the trachea or larynx. No suppurative or necrotic lesion was found in any case (Fig. 6). Aspiration of stomach contents was seen in 8 cases. In 3 this aspiration was massive and regarded as the cause of death, since it occurred under circumstances in which the infant was unable to free itself from a position of restraint (cases 14 and 15), or unable to dislodge an obstruction in the upper respiratory passages (case 6). When aspiration occurs in association with another and adequate cause of death, such as the presence of a lethal amount of carbon monoxide or extensive burns, it would seem reasonable to regard the aspiration as an agonal event, and purely incidental to the asphyxia-induced vomiting during the unconscious and moribund state.

OTHER ORGANS

Brain. Slight meningeal edema was noted in 2 cases of strangulation; in one case of asphyxia by compression of the chest; and in one case of carbon monoxide asphyxia. Occasional focal, usually perivascular, hemorrhages were noted, particularly in the cases of strangulation. The vessel walls and the extravasated erythrocytes in these involved areas were normal. Thrombi were never seen.

Adrenal Glands. Slight hyperemia was found twice in the adrenal glands. In no instance was hemorrhage noted.

Liver. In 8 cases occasional minute foci of lymphocytic infiltration were seen in the liver. As a rule these were smaller than those noted in the infants found dead. Sinusoidal congestion was not present. The hepatic cells usually contained abundant glycogen.

Kidney. Fifteen cases had minute foci of healed glomerulitis varying from the presence of a single hyalinized glomerulus to several groups of scarred glomeruli in one section. Active glomerulitis was not seen.

Submaxillary Gland and Testis. The submaxillary gland was available for study in 8 cases; 2 of these showed minute foci of lymphocytic infiltration. The testis was similarly involved in one of the 7 instances in which sections were taken. In the 3 cases with the lymphocytic infiltration described, there was also either gross or microscopic evidence of early incidental mastoiditis.

Lymphatic Tissues. In all but 2 infants the spleen contained large follicles with distinct borders and normally developing conspicuous germinal centers (Fig. 43 of Part I of this series[1]). Demarcation be-

tween the white and red pulp was distinct. In the thymus the Hassall's corpuscles were seen to contain far less nuclear débris than those of infants found dead. Hyperemia was seldom present, hemorrhages were rare, and thrombi were never seen. The cervical, axillary, bronchopulmonary, and peripheral lymph nodes usually had large well defined follicles. Hyperemia was observed in only 6 cases and, when seen, usually was slight: those dying of carbon monoxide asphyxia, burns, or of suffocation by live steam. In these cases the hyperemia was limited to the bronchopulmonary lymph nodes. Minute hemorrhage was encountered in only one instance, a case of suffocation by steam. Depletion of lymphocytes or sinus catarrh was not seen.

Bacteriologic Examination

In 17 of the 26 cases cultures were taken, usually from one or more of the following sources: tonsil, bronchus, lung, spleen, and heart's blood. Mastoids containing fluid were cultured in several instances. In 6 cases a mixed flora which we considered to be non-pathogenic was recovered. In 3 cases the organs cultured were sterile; in 2 of these only lung and spleen were cultured; in the third, lung, spleen, heart's blood, and cerebrospinal fluid were sterile and non-pathogens were recovered from the bronchus. In 3 cases hemolytic *Staphylococcus aureus,* and in 3 cases *Streptococcus haemolyticus,* was recovered. Two cases had both. These organisms usually were found in tonsil, tracheobronchial tree, or lung; and twice in the mastoid. The spleen and heart's blood in 6 of these cases were sterile. *Strep. haemolyticus* was recovered from the spleen twice; once when it was found also in the tonsil (case 3) and once when it was present in mastoid, lung, and heart's blood (case 16). As a result of these bacteriologic findings in the violent deaths we regard the mere recovery of pathogenic organisms to be of questionable significance.

Discussion

The lungs of the 5 infants who drowned were normal except for occasional minute patches of collapse found in the periphery. However, in 2 of the older infants (cases 13 and 22, eight and nine months old) there were lesions consistent with a diagnosis of focal interstitial pneumonitis. These changes were limited in extent. The absence of widespread pulmonary congestion, edema, hemorrhage, and collapse in the remainder of the lung readily distinguished these incidental lesions from the lungs of infants found dead. Since in some of the cases of drowning the post-mortem interval was as much as 18 to 24

hours, it may be concluded that congestion of the lungs in human material is not a post-mortem development as has been alleged.[2]

In the deaths due to causes other than drowning, more prominent, although varying, degrees of edema, collapse, and intra-alveolar accumulation of macrophages and lymphocytes were seen in all of the lungs. Congestion of slight to moderate extent was found in several asphyxial deaths including deaths from carbon monoxide. Hemorrhage was seen only rarely; it was always slight and usually limited to the intra-alveolar spaces. Extravasated red cells were well preserved and thrombi were never seen. The extent of the pulmonary changes seemed related to the rapidity of death and to the age of the subject. When death was slow there was more time for response of the lungs to the stimulus of anoxia. In older infants collapse was less marked, probably because of the better development of their lung structure.[3] A frequent response during even the brief period of agony in these infants was the focal accumulation of macrophages and lymphocytes within the alveoli, especially in association with edema and collapse. This microscopic appearance, when seen in sudden death, has recently been called "mononuclear pneumonia."[4] While it is true that these cells appear in varying numbers in the lungs of infants dying suddenly, this change may be equally prominent in many rapid deaths from violence.

Bronchial epithelial changes were found in those cases exposed to smoke in conflagration and live steam. It is well known that chemical and physical irritants may provoke a response in respiratory epithelium with great rapidity. Winternitz, Smith, and McNamara[5] have shown previously that changes in the lungs in chemical pneumonia were indistinguishable from those of influenza. Bronchial mural lymphocytic infiltration was encountered in 8 cases, 2 of which also had isolated foci of interstitial pneumonitis. In 4 cases the lesions were extremely minimal. All but one of these infants were over 7 months of age. The absence of changes indicating severe bronchial inflammation, such as hyperemia, mural edema, epithelial degeneration, or polymorphonuclear leukocytic infiltration, distinguished these cases of incidental mural bronchitis from those in which the inflammatory lesions of the bronchi were manifestations of fulminating respiratory disease.

The mastoid changes found in 7 cases were interpreted as evidence of recent respiratory inflammation, with the exception of the infant of case 3 who had survived extensive burns for ½ hour. The edema and eosinophilic infiltration found in the lining epithelium of the mastoid

in this case could readily have been a reaction to irritating smoke inhaled by the infant. Therefore, we did not consider it indicative of incidental respiratory disease. In this case, in addition, no lesions were found in any other region of the respiratory tract. In 5 of the 6 cases with early mastoiditis there were other changes seen in the respiratory tract. As already noted, in one case interstitial pneumonitis was found; in 4 others there was either mural bronchial or tracheal submucosal lymphocytic infiltration. In the sixth case in which serous fluid in the mastoid had been found at necropsy and for which there was a history of exposure to the mother with a cold, no sections of the upper respiratory tract were available for study. In 5 of these 6 remaining cases of microscopic mastoiditis the pathologic evidence for the presence of incidental respiratory disease was further supported by the clinical history. In one case there had been medical attention for a cold the day of death; one child had recent pertussis; one had been exposed to a mother with a cold; one had just recovered from a cold; and one (drowning, with interstitial pneumonitis) was reported to have been unusually fretful the day of death.

Since acute respiratory infection is so common in early life, it is not surprising that such incidental lesions were found in the mastoids, tonsils, bronchi, or lung. The mild character and limited extent of the lesions, as well as the absence of generalized visceral congestion, thrombosis, tissue edema, and changes in the lymphatic system, distinguished these tissues of infants dying of violence from those of infants found dead,[1] observed to die,[6] or dying after fulminating clinical infection,[7] in whom the gross necropsy findings were inconclusive.

Summary and Conclusion

Gross and microscopic studies of 26 infants, the majority under 1 year of age, dying immediately of violence, revealed the following findings:

The viscera of drowned infants most closely resembled the normal. In violent deaths from other causes focal pulmonary changes consisting of collapse, intra-alveolar edema, and accumulations of alveolar macrophages and mononuclear cells of varying degrees were noted. The pulmonary changes, however, were limited to the alveoli except in those infants dying in conflagrations or exposed to live steam in whom there was, in addition, bronchial epithelial injury.

Multiple, though minimal, lesions indicative of incidental respiratory disease occurred in 6 cases. In 7 additional cases there were single minimal lesions in the respiratory tract.

The absence of morphologic evidence of systemic reaction (vascular or lymphatic tissue) as well as the low incidence of minor respiratory lesions in infants dying immediately after violence is in sharp contrast to the histologic findings in infants dying suddenly while in apparent good health. This contrast establishes the pathologic significance of the microscopic findings we have described in the previous two papers of this series.[1,6]

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LEGENDS FOR FIGURES

FIG. 1. Case 2 (ME 47–12). Male, 17 days old. Drowning. Lung: Normal bronchus and surrounding parenchyma. × 40.

FIG. 2. Case 8 (ME 47–27). Male, 3 months old. Extensive burns. Lung: Agonal aspiration of stomach contents. Normal bronchial wall. Intra-alveolar edema and slight macrophage proliferation. × 40.

FIG. 3. Case 19 (ME 51–257). Male, 9 months old. Suffocation by live steam. Lung: Marked epithelial degeneration and desquamation. Minute patches of collapse are seen also. × 20.

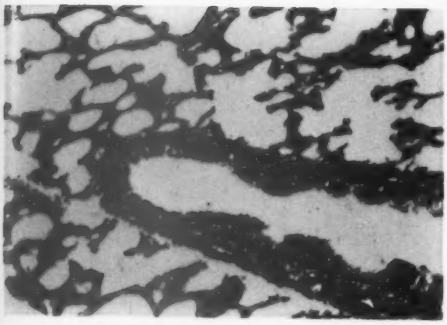
FIG. 4. Case 18 (ME 52–196). Male, 8 months old. Homicidal strangulation by rope. Lung: Slight collapse and presence of edema fluid and macrophages in the alveoli may be seen. There is no congestion despite a post-mortem interval of 20 hours. × 20.

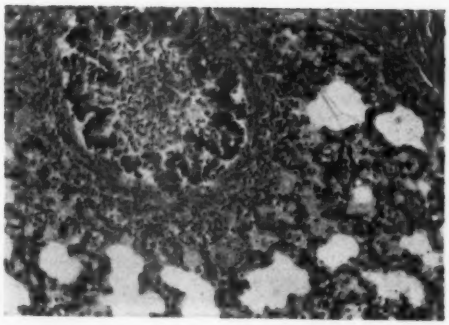
FIG. 5. Case 25 (42–A–72). Female, 18 months old. Asphyxia by blood clot surrounding impacted eggshell in larynx. Lung: Normal lung, well aerated alveoli, complete absence of congestion, alveolar macrophages, or edema. × 4.

FIG. 6. Case 24 (51–A–95). Male, 18 months of age. Asphyxia by compression of chest by overturned dresser. Vocal cord is normal. × 20.

FIG. 7. Case 6 (ME 43–143). Male, 3 months old. Aspiration of stomach contents following impaction of safety pins in pharynx and esophagus. Larynx shows normal epithelium and mucous glands, with no infiltration. Aspirated material is visible in the lumen. × 20.

FIG. 8. ME 52–273. Male, 7 months old. Accidental strangulation by harness. Cervical lymph node. × 9.





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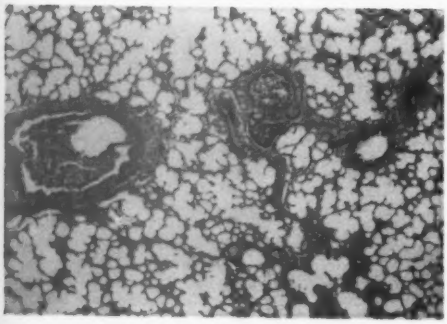

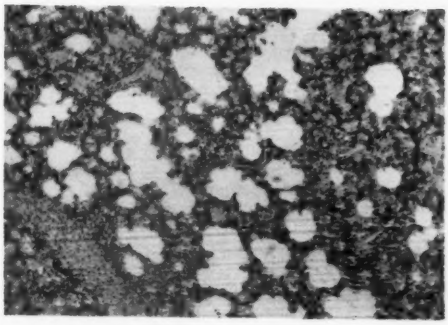

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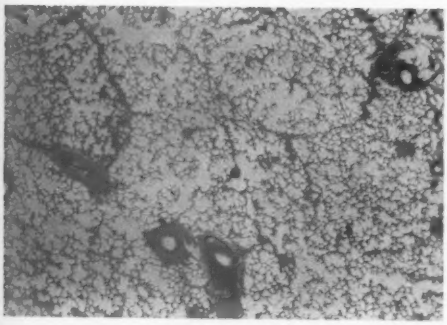

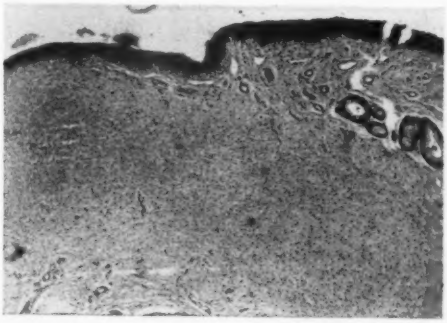

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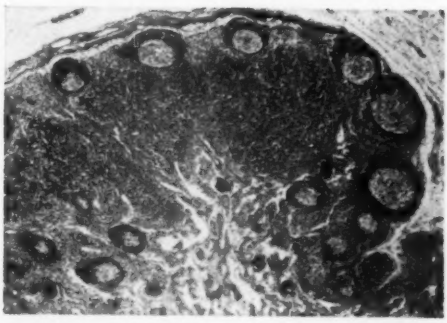

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FIG. 9. Case 13 (ME 42–65). Male, 8 months old. Drowning. Pale lung; no congestion. A few petechiae are seen.

FIG. 10. Case 18 (ME 42–196). Male, 8 months old. Homicidal strangulation by rope. Normal thymus, 60 gm. Normal adrenal glands. Petechiae are absent.

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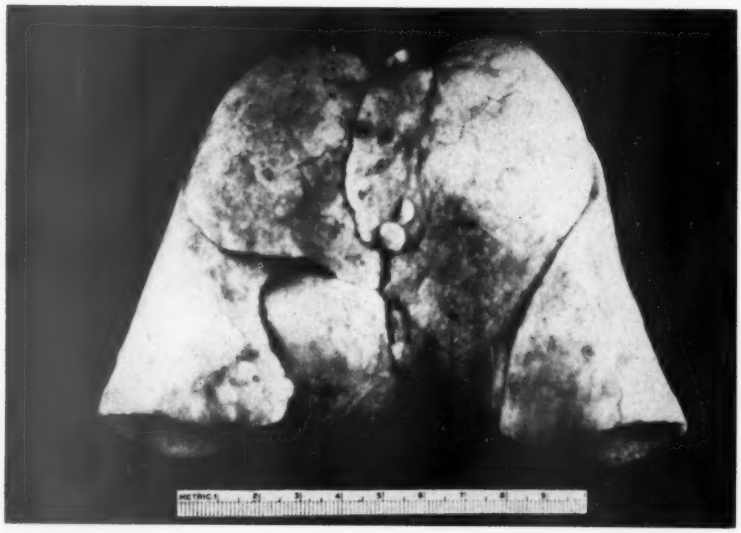

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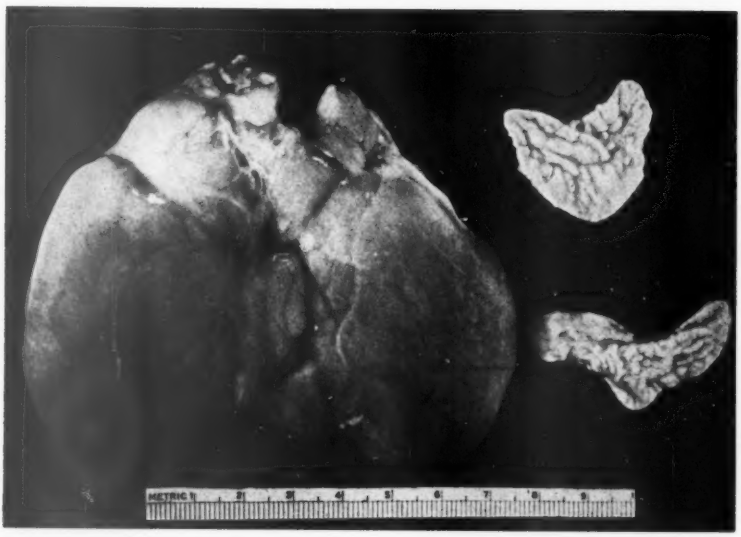

FIG. 11. Case 22 (ME 45–75). Male, 9 months old. Drowning. Lung: Interstitial pneumonitis, incidental finding. Remainder of lung was normal. × 205.

FIG. 12. Same case as that from which Figure 11 was taken. Lung: Residual organizing pneumonitis. The adjacent parenchyma is normal, with no congestion or edema. × 12.

FIG. 13. Higher power (× 110) of normal lung in periphery of Figure 12.

FIG. 14. Case 5 (ME 46–101). Female, 2½ months of age. Drowning. Lung: Slight collapse; a few alveolar macrophages and edema fluid are seen. This was present in only small areas in the periphery. × 110.

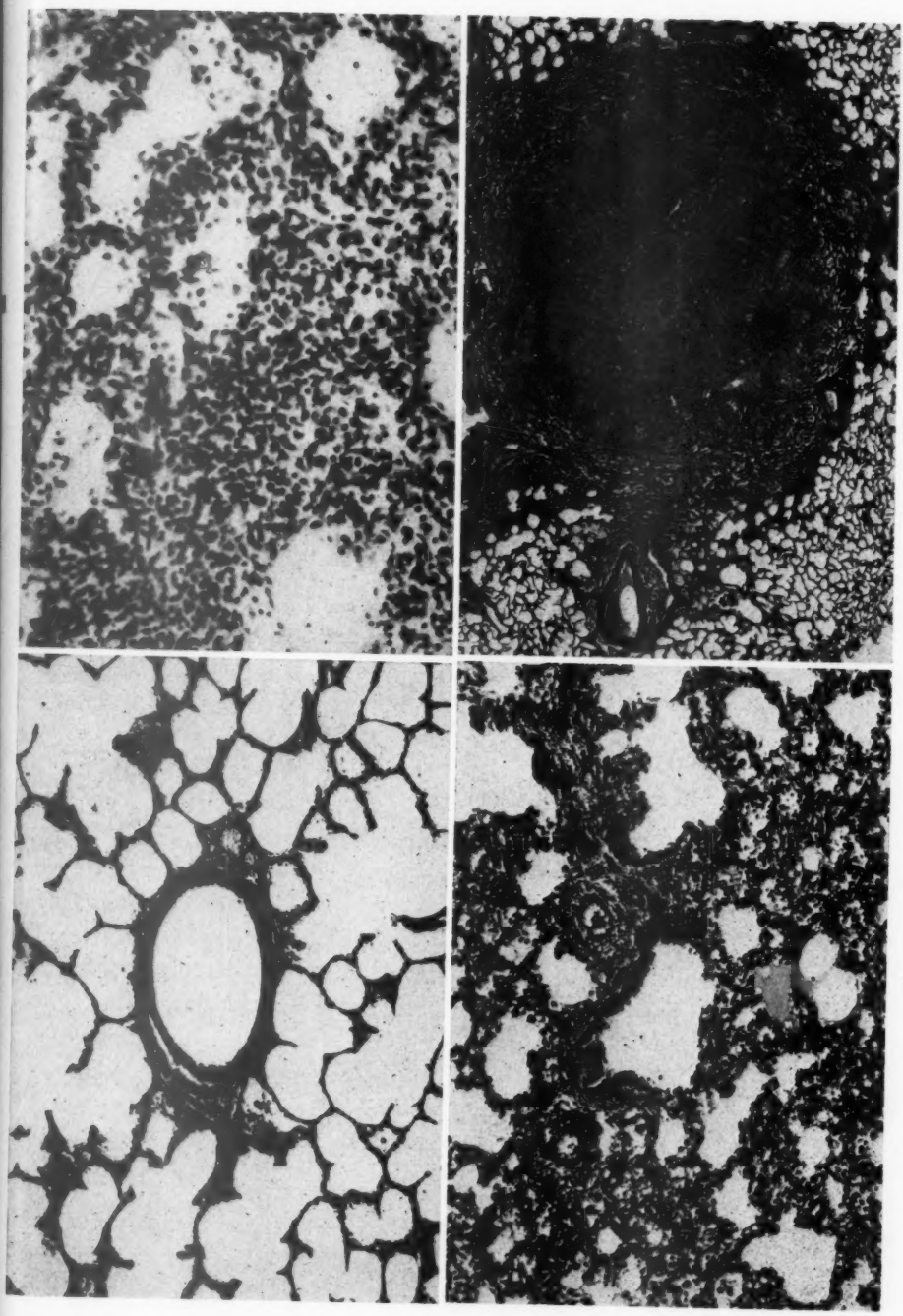

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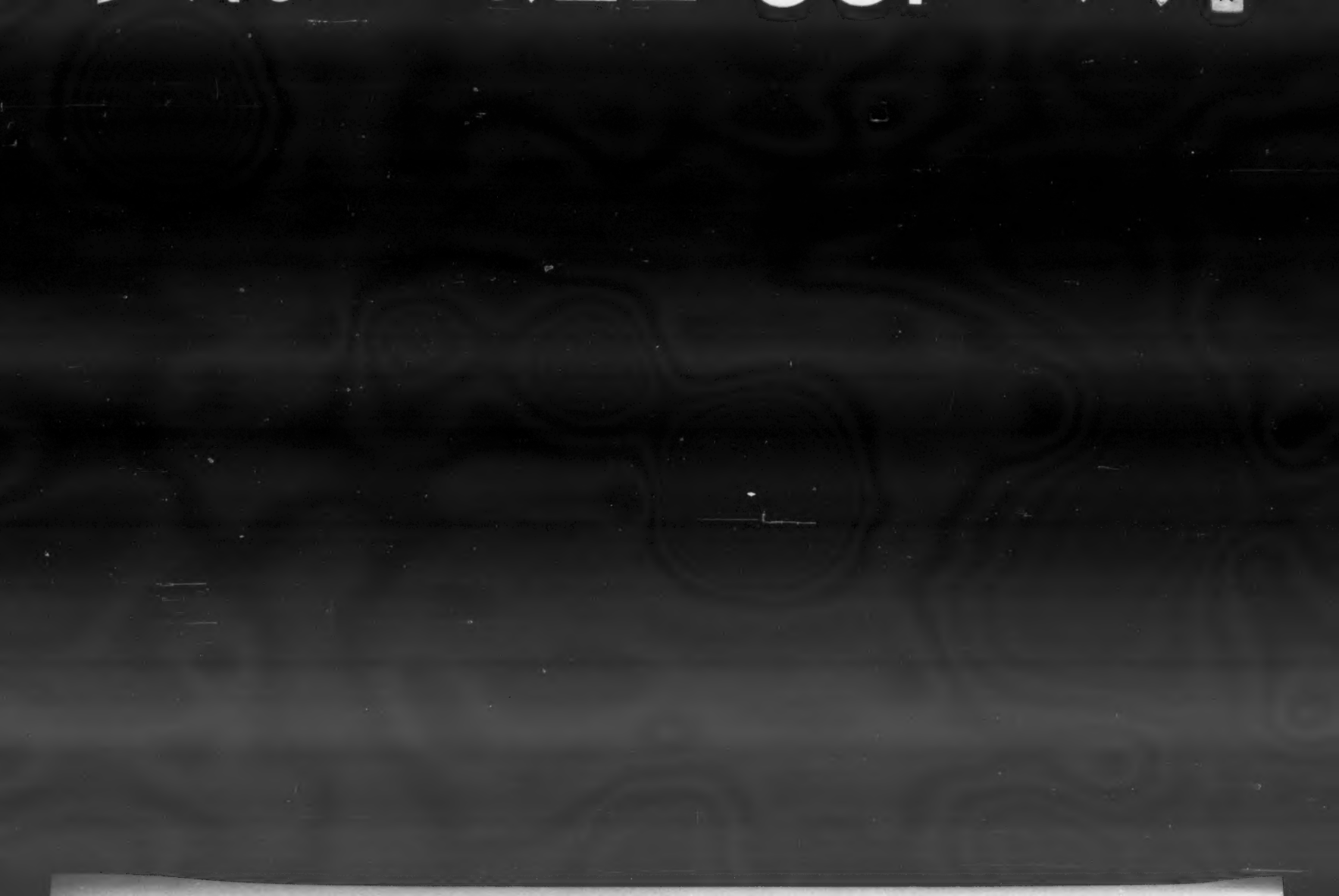
FIG. 15. Case 14. (ME 44–44). Female, 8 months old. Aspiration of stomach contents while in restraint. Lung: Of note are the intra-alveolar edema and the presence of intra-alveolar macrophages. No congestion. × 110.

FIG. 16. Case 8 (ME 47–27). Male, 3 months old. Extensive burns. Lung: Same changes as in Figure 17. × 110.

FIG. 17. Case 16 (ME 51–62). Male, 8 months of age. Carbon monoxide asphyxia. Lung: The congestion of alveolar walls is more marked than in other traumatic deaths, but is less prominent than that seen in the great majority of infants "found dead." × 110.

FIG. 18. Case 6 (ME 43–143). Male, 3 months old. Aspiration of stomach contents following impaction of safety pins in pharynx and esophagus. Lung: Intra-alveolar edema and macrophages; minimal congestion. × 110.



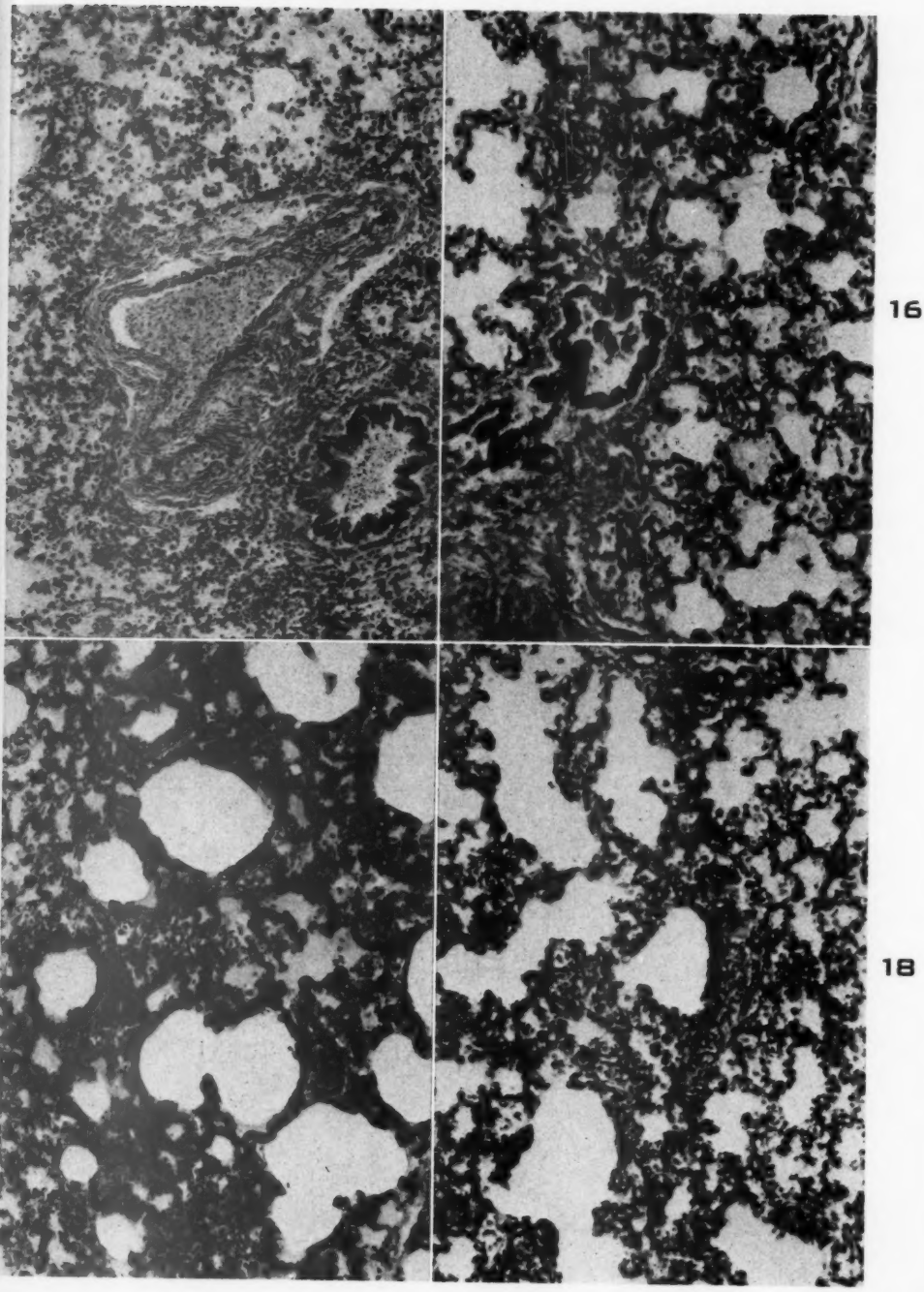


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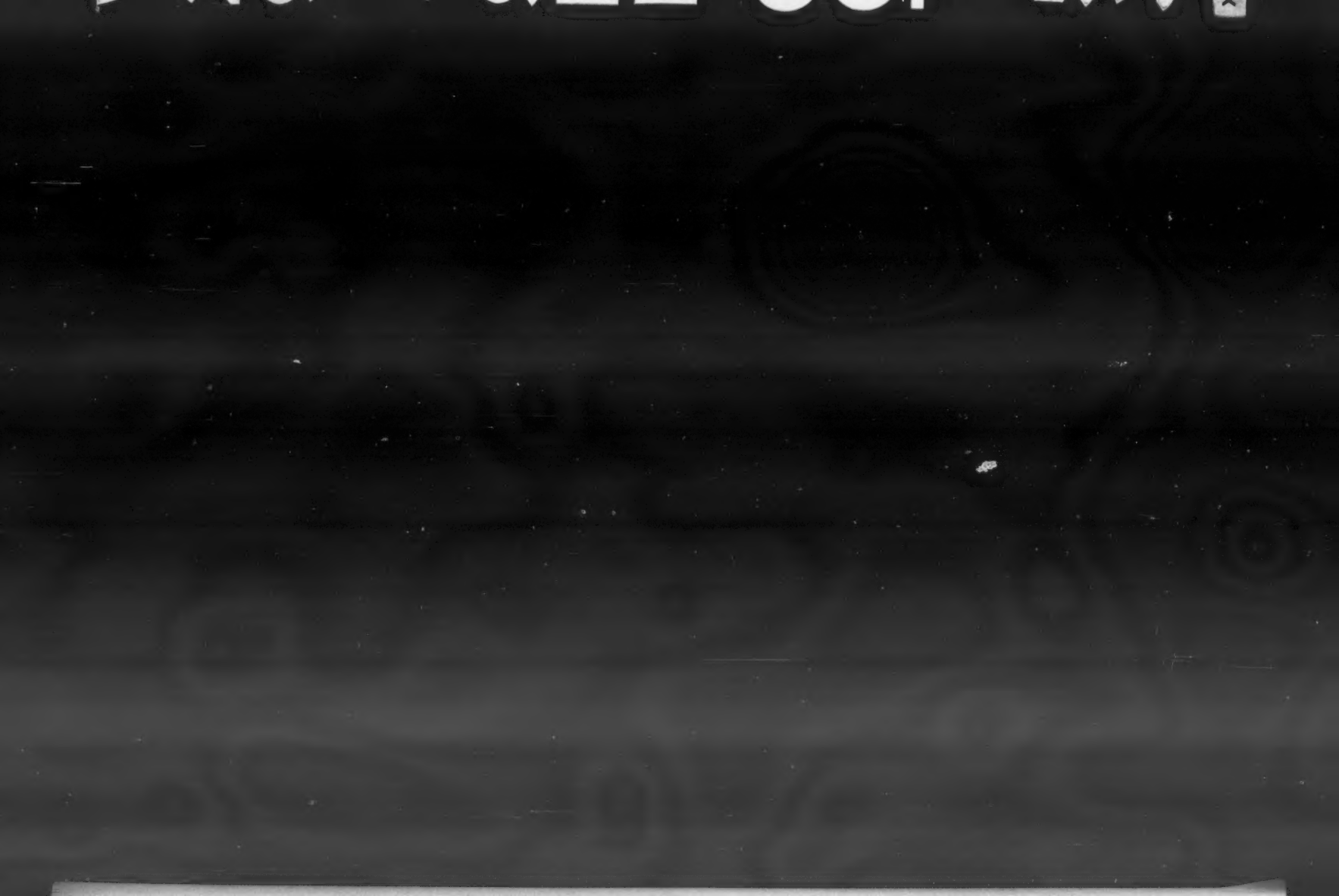
FIG. 19. Case 23 (ME 50–144). Male, 16 months old. Asphyxia by aspiration of lollypop. Lung: Intra-alveolar edema, macrophages, and lymphocytes. × 460.

FIG. 20. Case 24 (51–A–95). Male, 18 months old. Asphyxia by compression of chest by overturned dresser. Lung: Of note are the intra-alveolar edema and the presence of macrophages. This was seen only in focal areas. × 460.

FIG. 21. Case 5 (ME 46–101). Female, 3½ months of age. Drowning. Lung: Intra-alveolar macrophages (high power of Fig. 14). Absence of congestion in the alveolar capillaries in Figures 19, 20, and 21 is to be noted when compared to Figures 46 to 48 of Part I.[1]



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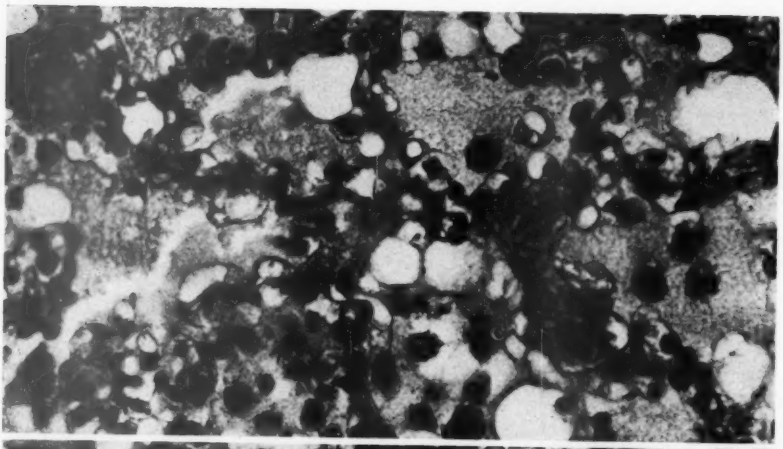

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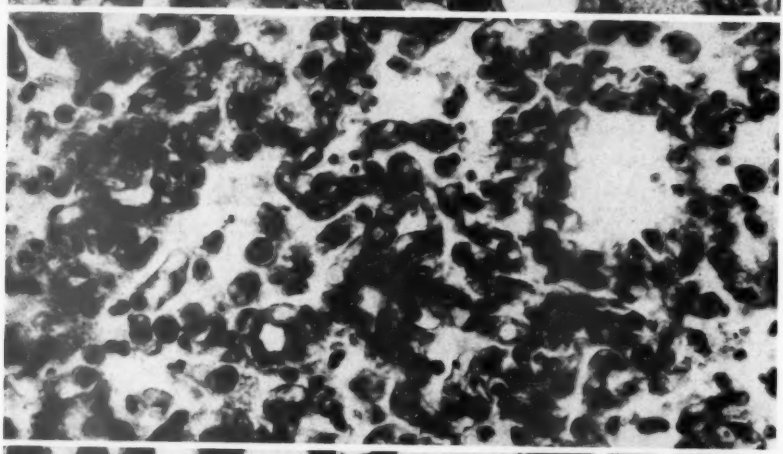

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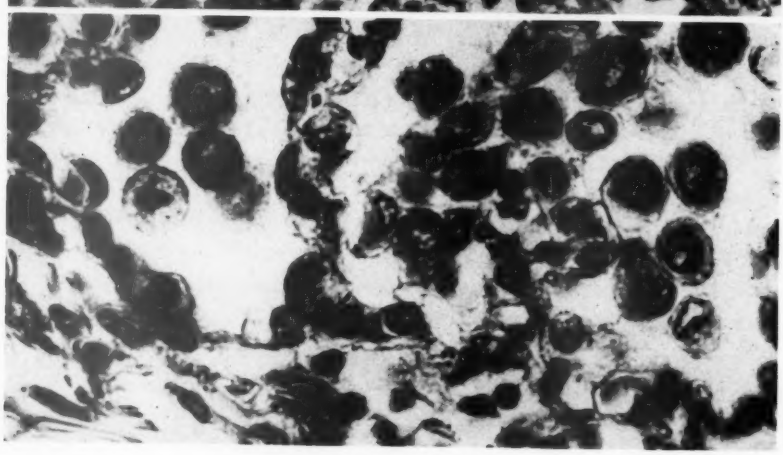

OVARIAN RETE CYSTS *

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Cysts of the ovarian hilus have been produced in experimental animals, but have not received much attention in human beings.[1] The normal human ovary has a cluster of small tubules and foci of interstitial cells resembling Leydig cells of the testis, both located in ovarian medullary tissue in the rete region where the mesovarium is attached (Fig. 1). Earlier embryologic opinion was that rete tubules were homologous to the male vas deferens, but according to Gillman[2] rete cords in the female are essentially sex cords developing from coelomic epithelium and not of mesonephric (wolffian duct) origin.[3]

In a review of ovaries from 740 women, obtained at necropsy, ovarian rete cysts were identified in 25 (3.4 per cent). Most of these cysts were small, and only 5 were described grossly as filled with clear fluid. These measured 0.4 to 2 cm. in diameter. Others were recognized microscopically. The site of origin in the abundant, tough, medullary, fibrous tissue of the rete would tend to interfere with the enlargement of rete cysts to sizes which could be recognized clinically.

Microscopically, most of the rete cysts ordinarily would be considered as "simple cysts." Their anatomical location and the resemblance of lining epithelium to the normal rete tubules served to distinguish this specific group. The epithelium was at times composed of tall columnar cells crowded together, with basal nuclei and with bulbous, irregular, free margins. In their lack of smooth, regular, cytoplasmic margins and arrangement they resembled wolffian urogenital epithelial structures rather than müllerian derivatives. Also, when stretched, the cells lacked a regular epithelial polarity and size, but instead showed variable size, overlapping or stratification, and rounded cytoplasmic projections such as characterize mesothelial, mesonephric, or paraovarian cysts. Simulation of the epithelium in immature human testicular tubules, rete of adult testes, and areas in some adenomatoid tumors of epididymis was observed also (Figs. 2 and 3).

The underlying stroma was composed of ordinary collagenous fibrous tissue. In some instances foci of calcification or ossification were observed (Fig. 4), similar to changes observed in the vas def-

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erens, particularly in diabetes mellitus.[4] Comparable stromal metaplasias also are observed in some retroperitoneal neoplasms believed to originate from the urogenital ridge.[5]

The term ovarian rete cysts has been used mostly in animal pathology. It avoids the possible ambiguity of the term hilar cyst, since hilus cells of the ovary may refer also to the interstitial cells of Leydig type found in the ovarian hilus.

The 25 women with these cysts were from 27 to 84 years old, but all except 6 were over 55 years of age. In 5 cases the rete cysts were bilateral. In 8 of the women there was also ovarian cortical stromal hyperplasia[6] present. Each of 2 patients had had one ovary removed surgically, and in another instance parts of both ovaries had been resected.

Eleven patients had cancer, including 2 cases of cervical and 2 of mammary carcinoma. There were also one case each of adenocarcinoma of endometrium, gallbladder, colon, and lung, one case of gastric carcinoma simplex, one of chronic leukemia, and one of lymphosarcoma. Five other patients had diabetes mellitus, 3 with hyalinized pancreatic islets. Seven had histories of hypertension, and 5 were obese. Two had biliary cirrhosis and one, cardiac cirrhosis secondary to rheumatic heart disease.

Hyperplastic changes were observed in some endocrine organs of 20 cases, usually limited to glands of one or two types. Adrenal cortical hyperplasia was found in 6 women and a demarcated adrenal adenoma in another case. Four others had abnormally nodular adrenal cortical architecture. In the pancreas, islets of Langerhans were increased in size and number in 6 instances. Papillary or adenomatous pancreatic duct hyperplasia was observed in 7 cases. Adenomatous goiter was found in 5 women, and 2 had diffusely hypertrophied glands. One thyroid fetal adenoma was identified. Parathyroid glands were examined in 6 of the necropsies. One mixed chief and oxyphil-cell adenoma was found, without osteitis fibrosa cystica; and 2 cases had oxyphil nodules identified.[7] One thymus was enlarged.

Two of three pituitary glands examined showed an adenomatous increase of eosinophils, and in one gland the basophils also were hyperplastic. This latter patient was a 62-year-old diabetic woman with obesity, nodular adrenal cortical hyperplasia and adenoma, hypertension, hypertrophy of the thyroid gland, and hyperostosis frontalis interna. Evidently she represented an example of Morgagni's syndrome[8] of hyperpituitarism.

Endometrial polyps were observed in 5 cases, one also with endometrial carcinoma. Four other women had endometrial hyperplasia, usually of cystic pattern. In 5 uteri, leiomyomas were found. Intestinal polyps were present in 3 cases, including 2 polyps of colon and one of duodenum.

Masculinization, with facial hirsutism or partial baldness, was present in one case each. One woman had Paget's disease of calvarial bone.

Discussion

A majority of the women with ovarian rete cysts had morphologic evidences of endocrine imbalances. The abnormalities were not of any one particular type, but took the following patterns:

(1) Hyperestrinism, with endometrial polyps or cystic hyperplasia, 9 cases.[9] In 4 of these, ovarian cortical stromal hyperplasia was present. Two others with atrophic ovaries had hepatic cirrhosis, and 2 demonstrated abnormal nodularity of adrenal cortices. The remaining case had had one ovary removed surgically.

(2) Gynecologic operations, 4 cases. Three patients had either one ovary removed, or parts of both ovaries resected. One had a supracervical hysterectomy.

(3) Masculinization, 2 cases. It is likely that other instances were overlooked, including the woman with Morgagni's syndrome. Adenomatous hyperplasia of rete tubules and stroma without cyst formation has been observed with arrhenoblastoma.[10]

(4) Adrenal cortical hyperplasia or adenoma, 7 cases. Four of these women were obese, and 3 had diabetes mellitus. The nodular hyperplasia involved chiefly the zonae fasciculata and reticularis.

(5) Hyperpituitary function, shown by hyperplasia of multiple endocrine glands and adenomatous pituitary architecture, 20 and 2 cases, respectively. The most frequent combinations were ovarian cortical stromal or pancreatic islet hyperplasia with adrenal cortical hyperplasia, 4 cases each. Obesity, diabetes, adrenal cortical hyperplasia, and thyroid hypertrophy occurred together in 2 women.

(6) Local factors, 3 cases. The remaining cases of this series lacked the above changes, but had abnormalities of pelvic circulation. In 2 cases this interference with blood supply was secondary to carcinoma of the cervix, and in one to regional ileitis. Perhaps local pressures occasionally may have predisposed mechanically to the formation of rete cysts.

In the series of human cases, etiologic factors suggested for rete

cysts corresponded closely to those already found in experimental animals.

(1) Estrogenic hormone injections have led to the development of ovarian hilar tubules, to cysts, or to adenomas in guinea-pigs, rabbits, or rats given large doses of estrin for at least 3 weeks.[11] Rats treated from early life with estrone benzoate, with or without equine pituitary extract, had these proliferations and also "syncytial tubules" resembling those of sterile testes.[12] Estradiol benzoate or propionate, or diethylstilbestrol similarly were effective in female guinea-pigs, their use being accompanied by a hyperplastic growth of ovarian germinal epithelium and some neoplastic derivatives.[13,14] Implantation of testes into the necks of newborn, littermate, female mice was followed after 250 to 408 days by formation in their ovaries of rete cysts up to two thirds the size of the ovarian cross section. Pfeiffer[15] attributed these cysts and other abnormalities observed to constant endogenous estrogen production by the persisting immature testicular implants.

(2) Surgical fragmentation of ovaries in guinea-pigs has been reported by Lipschutz[16] to lead to a development of rete cysts in the ovarian remnant, and masculinization indicated by hypertrophy of the clitoris.

(3) Testosterone propionate treatment of newborn female rats for 30 days or ante-natal and post-natal testosterone in mice had similar effects on both the ovarian rete and clitoris.[12,17]

(4) Adenomatous adrenal cortical nodules followed intrasplenic ovarian grafting in ovariectomized mice, associated with neoplastic proliferations of the granulosa cells.[18] Tubular adenomatous rete nodules also were present, and the illustration provided by Furth and Sobel[18] resembles those of Lipschutz.[16]

(5) Intrasplenic implantation of ovaries is a further experimental technique observed to produce abnormal medullary tubules and nodular hypertrophic rete growths of tubules and stroma.[16] Induced ovarian-hypophysial imbalance in this situation is generally considered to involve a functional hyperpituitarism. It is not unlikely that the other experimental techniques depended also partly on induced pituitary dysfunction for their effectiveness in producing rete cysts.

Another morphologic stigma of hormonal imbalance is provided by the recognition of human ovarian rete cysts. They are not specific for any single hormonal abnormality, but are found in women who have endocrine dystrophies which appear to be similar to the experimental conditions leading to ovarian rete cyst formation in animals.

Summary

Twenty-five human cases of ovarian rete cysts are reported. Only 5 were recognized macroscopically. Others were microscopic and lined with an epithelium characteristic of rete tubules. Women with these cysts usually had other tissue alterations at necropsy, classifiable as evidences of hyperestrinism, adrenal cortical dysfunction, or pituitary hyperfunction. Two women showed masculinization. Four had previous gynecologic operations. These are the same conditions found to be associated with ovarian rete cyst or adenoma formation in experimental animals.

Appreciation is expressed to Drs. Arthur T. Hertig and Shields Warren for their suggestions and criticisms.

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LEGENDS FOR FIGURES

FIG. 1. Normal cluster of rete tubules in the ovarian hilus of a woman 56 years old. Hematoxylin and eosin stain. × 250.

FIG. 2. Rete cysts from the ovary of a 38-year-old woman who died of uremia secondary to carcinoma of the cervix. The cysts were bilateral. Of note is the abundance of interstitial hilus cells. Hematoxylin and eosin stain. × 150.

FIG. 3. Ovarian rete cysts, lined by flattened or columnar epithelium, from a woman 60 years old who died of cardiac failure. Cardiac cirrhosis, a mucosal polyp of the colon, and hyperplasia of pancreatic ducts and endometrium were present. Eosin and methylene blue stain. × 125.

FIG. 4. Calcification of the wall of a rete cyst from the ovary of a 63-year-old patient, dying of pulmonary embolism following resection of a colonic carcinoma. Prominent adrenal zona reticularis, hyperplasia of ovarian cortical stroma, and endometrial cystic hyperplasia were observed also. Eosin and methylene blue stain. × 250.

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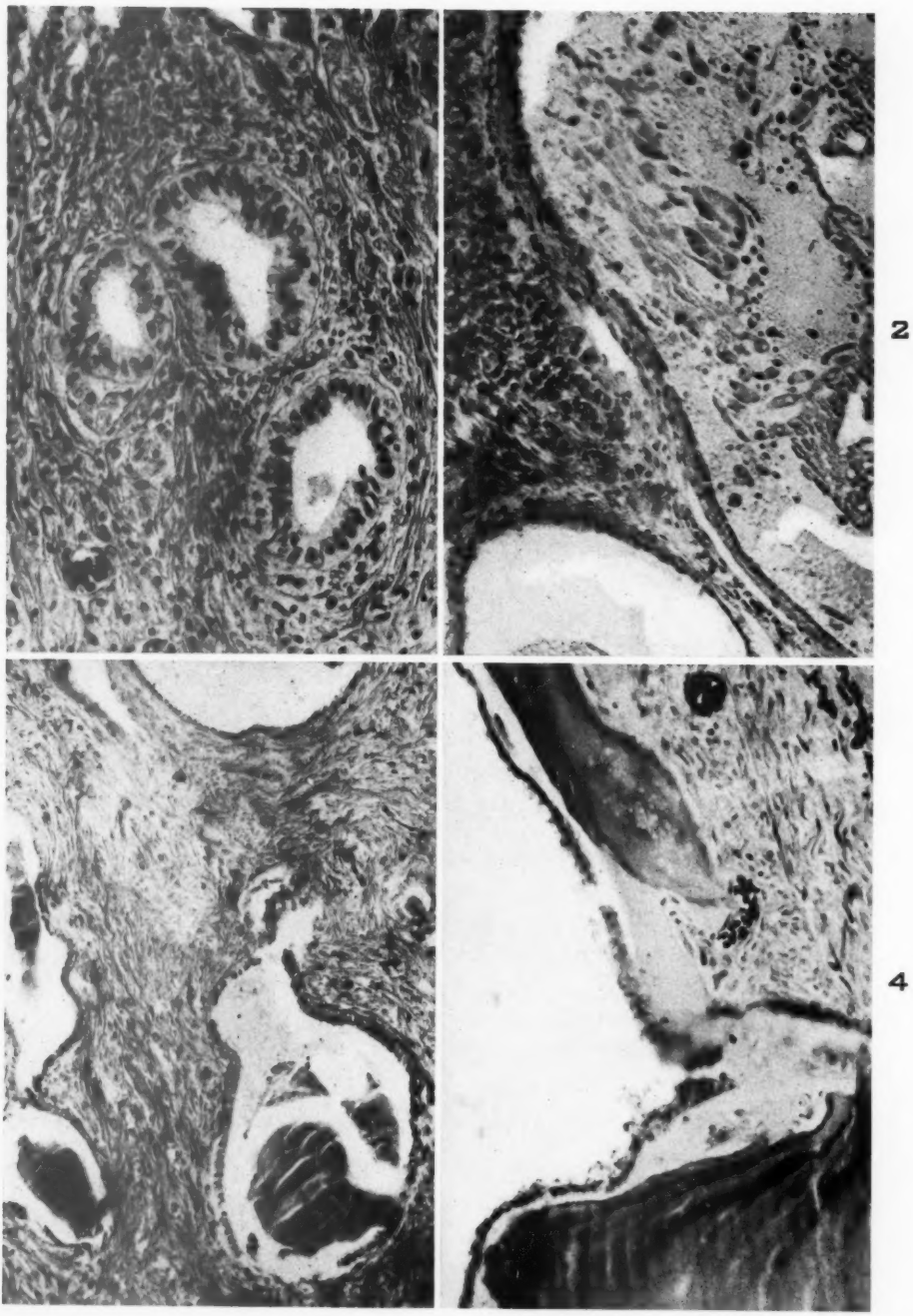
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OBSERVATIONS ON HUMAN TISSUE CULTURES NATURALLY INFECTED BY HISTOPLASMA CAPSULATUM *

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Our interest in the study of *Histoplasma capsulatum* in tissue culture was first stimulated by experimental observations on supposedly normal horse amnio-allantoic membrane and adult horse tissue maintained *in vitro*.[1] In certain of the stained preparations, intracellular yeast cells morphologically identical with *H. capsulatum* were observed. Subsequently,[2] it has been established that chick and horse tissues maintained *in vitro* could be infected experimentally with the yeast-cell phase of *H. capsulatum* with development of numerous forms quite comparable to those of the natural disease.

In the present paper it is deemed of interest to report studies on known and suspected cases of naturally occurring histoplasmosis by tissue culture methods.

MATERIALS AND METHODS

Tissues

Splenic tissues from two different sources were used in this study. In one instance portions of spleen were removed aseptically at necropsy 6 hours after death from a child who had died of disseminated histoplasmosis. The other case was that of a 6-months-old female ill with a chronic debilitating disease on whom splenectomy was performed. The preoperative diagnosis was Letterer-Siwe's disease or histoplasmosis. Prior to surgery repeated cultures of the blood and bone marrow were negative. However, a culture of the bone marrow made several days before showed a few colonies of the fungus several days after the operation. Cultures of the spleen at room temperature and at 37° C. yielded a very few colonies with typical cultural characteristics of *H. capsulatum*. The histoplasmin and complement-fixation tests were negative. The remarks of the pathologist are of interest: "The severity of the preoperative clinical picture and degree of splenomegaly are interesting in view of the extreme paucity of organisms in the histological sections."

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The several tissues were minced and stored separately in Earle's balanced salt solution at 4° C. until ready for use some hours later.

Media for Culture

The nutrient fluid for the Maitland (flask) method consisted of 40 per cent sterile filtered horse serum and 60 per cent Earle's salt solution.[3] The liquid medium for the plasma clot cultures was composed of 40 per cent horse serum, 57 per cent Earle's salt solution, and 3 per cent chick embryo extract. Chick embryo extract and chicken plasma were prepared by conventional methods.

Sterility of all materials was determined by cultures in thioglycollate medium at 37° C.

Techniques of Cell Cultivation

The several tissues used for the two methods to be described[4] were minced into fragments averaging 2 to 3 mm. in width and rinsed three times with Earle's salt solution.

Plasma Clot Method. Three or four pieces of tissue were imbedded in plasma clot (equal parts of chicken plasma and embryo extract) on a no. 1 coverglass, 12 by 50 mm. The preparation was then placed in a sterile, rubber-stoppered, pyrex test tube, which measured 16 by 15 mm. After standing for 1 hour, 2 ml. of nutrient fluid was added to each tube. All tubes were slanted at an angle sufficient to cover the slide and incubated at 37° C.

Flask Method. Ten to fifteen pieces of tissue with a minimum amount of fluid were transferred with a 5 ml. pipette to a 50 ml. rubber-stoppered Erlenmeyer flask containing 3 ml. of nutrient fluid at pH 7.6 and incubated at 37° C.

Experimental Findings

The primary purpose in attempting the experiments to be described was to make morphologic observations of the natural disease under conditions which would allow host cell and intracellular parasite to develop under as natural conditions as *in vitro* maintenance would allow. Secondarily, it was believed that information might be obtained as to how yeasts pass from cell to cell.

Observations on Tissue Removed Surgically

Plasma Clot Method. Ten preparations were examined daily for pH fluctuations and microscopic changes. Nutrient fluid was changed once weekly, remaining clear. Tissues were fixed *in situ* on the cover-

glass with Zenker-acetic acid fluid and stained with hematoxylin and eosin after 4, 5, 6, 10, 13, and 20 days, respectively, of incubation. Initially, migrating leukocytes and masses of erythrocytes obscured the scanty outgrowth of fibroblast-like cells which was first noted on the third and fourth days, with some liquefaction of the plasma. The latter change was quite marked on the fifth day with retraction of the parent explant to one side, leaving bare glass spotted with a few adherent cells and surrounded by a thin peripheral ring of proliferating cells of the parent explant. The appearance of the cells on glass was variable, some being rounded and necrotic, while others, as in Figure 1, were elongated, with relatively healthy appearing cytoplasm and nuclei. A rare cell of the parent explant extending into the surrounding plasma, and a very rare cell remaining on the glass, contained yeast cells. Preparations fixed and stained after 10, 13, and 20 days of incubation showed progressive increase in the number of cells growing on glass and in the supporting clot. Concomitant with the growth of cells there occurred an increasing number of intracellular yeasts in both situations. Earlier cultures up to 20 days of incubation unquestionably showed more parasitized cells in the area imbedded in plasma clot. However, in the oldest cultures (20 days), it is a question as to which cells contained more yeasts—those growing on glass, or those in the parent explant. Many yeast-containing cells growing on glass were rounded and appeared necrotic; other cells on glass out of contact with plasma, appeared to be normal, with pale-staining cytoplasm and elongated cellular processes, in spite of the large content of yeast cells. (Figs. 2 and 3 illustrate such phenomena.)

In another 20-day-old culture (Fig. 4) it was interesting to note the presence of yeast cells in association with mitotic figures. The possible significance of this will be discussed.

Flask Method. Six flasks were prepared in the manner described. After 4 days of incubation at 37° C. the pH of the nutrient fluid had fallen from 7.6 to 6.8, requiring replacement. At the end of 7 days of incubation, tissues were fixed in Zenker-acetic acid fluid and stained with hematoxylin and eosin. Reference to Figure 5 will show that many large cells (reticulo-endothelial cells) contained numerous yeast cells. This is the more striking when the microscopic picture of the control sections, as noted by the pathologist, is recalled. The cultured tissues were loosely arranged and practically free of red blood cells and leukocytes, making the parasitized cells extremely easy to recognize. No visible necrosis could be detected.

Observations on Tissues Removed at Necropsy

Cultures of spleen removed at necropsy were studied by similar methods. Control sections stained with hematoxylin and eosin showed a massive infection of splenic cells. Nutrient fluid from 4-day-old cultures, and thereafter from both types of cultures, was very cloudy owing to the content of yeast cells. Fixed and stained preparations incubated for 7 days showed heavy parasitization of cells and extensive necrosis. A few scanty cells were observed around several of the explants in plasma clot. Cultures were abandoned at this point because of the marked stigmata of degeneration. The viability of tissue cells probably was affected by conditions of necropsy.

Comment

The results outlined indicate that host cells and yeast cells proliferate together in plasma clot cultures. Early cultures revealed the majority of parasitized cells to be confined to the area of the parent explant. A very rare cell remaining on glass after liquefaction of the supporting clot contained yeasts. Older cultures revealed numerous cells growing on glass to be infected, as well as the cells remaining in the plasma. It is reasoned from the evidence at hand that infected cells extend out on the glass from the parent explant and that probably the majority of infected cells are from this source. No doubt some infected cells remaining on glass after liquefaction of the clot early in the life of the culture contribute also. Do these observations indicate that yeast cells are spread from tissue cell to tissue cell by cell division? The presence of intracellular yeast cells in association with mitotic figures suggests that such may be the case.

Another observation relative to this subject should be considered. We have noted[5] in experimentally infected tissues that heavily parasitized cells disintegrate, liberating yeast cells. Are new cells then infected by the process of phagocytosis? In the cultures maintained for 3 weeks, even though many cells were distended with yeasts, all of them appeared intact and no yeast cells were found free in the clot.

Numerous intracellular forms were demonstrated also by the Maitland method. This technique is very simple to perform and might be useful as a method of diagnosis.

Summary

It has been shown that host cells and yeast cells proliferate together in tissue culture. The most striking feature was the great increase in intracellular yeast cells in the fibroblast-like cells growing on glass.

Some evidence was adduced that yeast cells are spread from tissue cell to tissue cell by cell division.

The methods employed may be useful in diagnostic problems concerned with infectious diseases.

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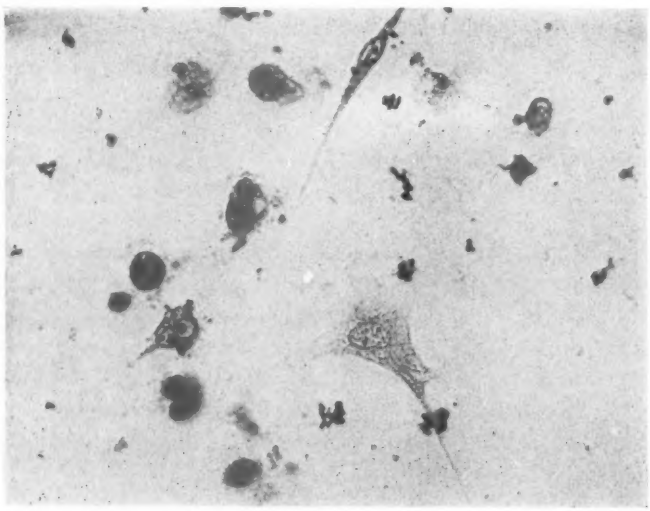
[*Illustrations follow*]

LEGENDS FOR FIGURES

FIG. 1. Five-day-old culture (plasma clot method) of spleen removed surgically, fixed in Zenker-acetic acid fluid and stained with hematoxylin and eosin. Of note is the varying appearance of cells remaining on glass following liquefaction of the clot. No intracellular yeasts are apparent. × 200.

FIG. 2. Twenty-day-old cultures from the same source as that for Figure 1 and cultured by the same method, fixed in Zenker-acetic acid fluid and stained with hematoxylin and eosin. The large, central area devoid of plasma clot is covered with cells growing on glass. The dark-staining, individual, rounded cells can be easily contrasted with the clumps of pale-staining, relatively healthy appearing cells as illustrated further in Figure 3. × 50.

FIG. 3. Same preparation as for Figures 1 and 2, illustrating well preserved cells filled with yeasts. The nuclei of several of the infected cells are quite eccentric. × 300.



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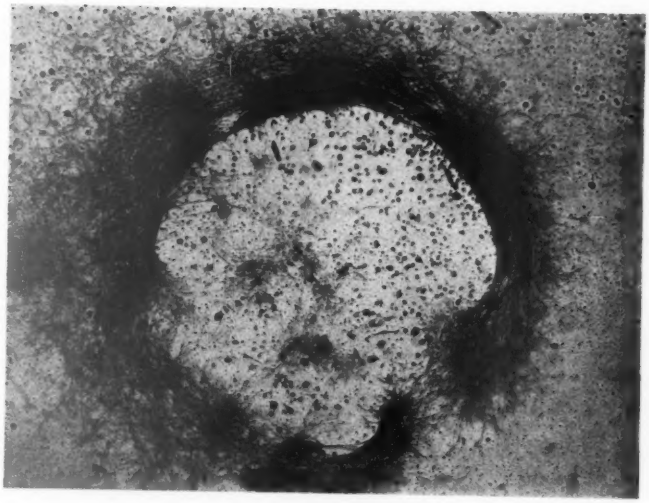

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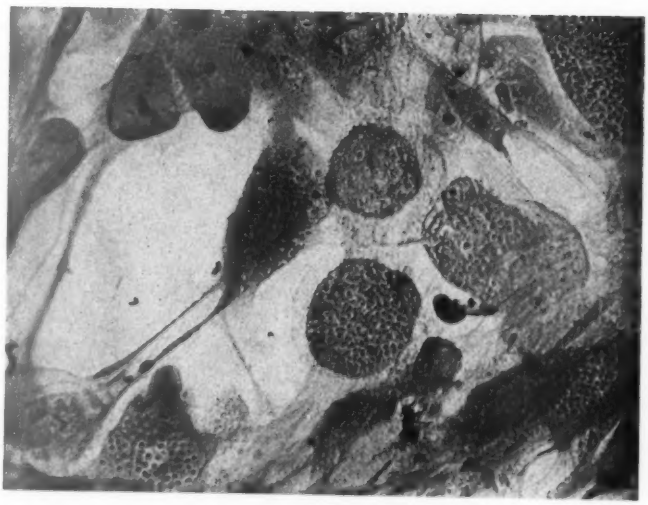

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FIG. 4. Another 20-day-old culture, from the same source as the preceding illustrations, fixed with Zenker-acetic acid fluid and stained with hematoxylin and eosin. Of note is the giant cell with numerous intracellular forms. The association of yeast cells with mitotic figures is especially interesting. One cell in mitosis is free of yeast cells. Drawing of photomicrograph. × 675. The drawings were made to eliminate undesirable débris.

FIG. 5. Seven-day-old cultures (flask method) of spleen removed surgically, fixed in Zenker-acetic acid fluid, and stained with hematoxylin and eosin. Numerous large cells, supposedly reticulo-endothelial cells, are filled with the yeast cell phase of *Histoplasma capsulatum*. × 150.

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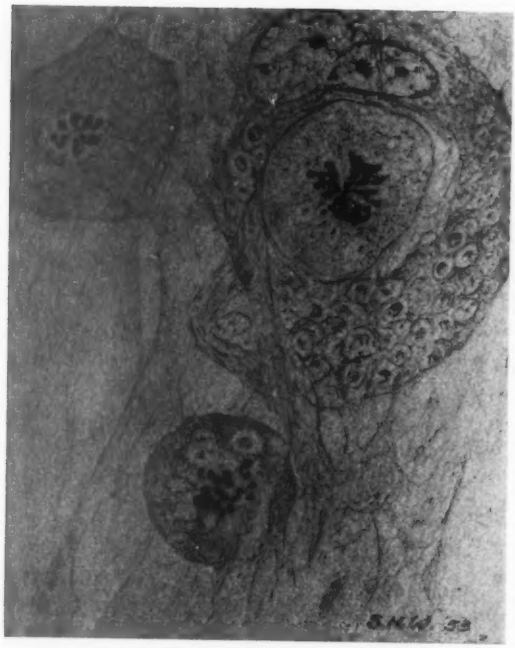

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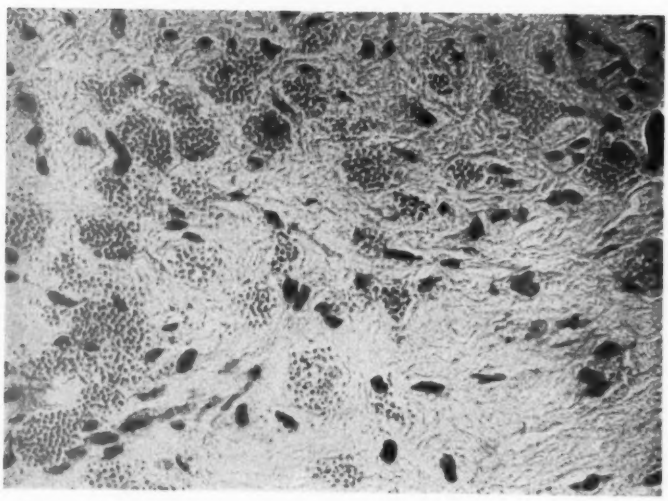

THE ROLE OF MUCOPOLYSACCHARIDES IN THE PATHOGENESIS OF INTIMAL FIBROSIS AND ATHEROSCLEROSIS OF THE HUMAN AORTA *

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A voluminous literature has dealt with the rôle of lipid deposition in the pathogenesis of atherosclerosis, but in recent years relatively little attention has been paid to structural changes in the vessel wall which might throw light on the essential nature of this process. Duff and McMillan[1] remarked on this in their splendid review when they made the amusing comment that one might wonder whether some authors conceived of an atherosclerosis so independent of the substrate of the vessel wall that it might occur in the absence of the blood vessels themselves.

In the past many of the German school emphasized that alterations in elastic tissue, collagen, and mucoid ground substance might be of the utmost importance in the development of atheroma. Wilens[2] recently has suggested that diffuse intimal fibrosis may be an early but integral part of the atherosclerotic process and noted that it was more pronounced in those areas susceptible to plaque formation. Rinehart and Greenberg[3] re-awakened interest in the rôle of the intercellular metachromatic substance in their report on the production of arteriosclerosis in pyridoxine-deficient monkeys, while more recently Moon and Rinehart[4] again have emphasized the possible rôle of mucopolysaccharides in their study on the histogenesis of coronary arteriosclerosis.

The present study deals with the human aorta, particular attention being paid to the relationship between the elastic fibers and the intercellular substance. It is emphasized that the tissue examined was taken mainly from areas which showed no gross atheromatous plaques, other than occasional small fatty streaks, in the hope that changes might be found there which would throw some light on the accumulation of lipids so frequently stated to be the basic lesion responsible for the development of these plaques.

* Received for publication, February 24, 1953.

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Material and Methods

The tissue was obtained from routine necropsy material, 124 blocks being examined from 88 cases. The age distribution and sex are tabulated in Table I.

A majority of the specimens were taken from the ascending aorta, though frequently a block from the descending thoracic aorta was included for comparison. The tissues were fixed in 10 per cent neutral formol-saline solution for 18 to 24 hours, and then embedded in paraffin. Serial sections were stained with hematoxylin and eosin, 0.5 per cent aqueous solution of toluidine blue, Verhoeff's elastic tissue method

Table I

Distribution as to Age and Sex of the 88 Cases Used in the Present Study

Age in years	Male	Female	Total	Age in years	Male	Female	Total
0–10	5	6	11	51–60	4	4	8
11–20	2	5	7	61–70	15	7	22
21–30	2	1	3	71–80	12	2	14
31–40	1	5	6	81–90	3	2	5
41–50	8	3	11	91–100	1	0	1
Total					53	35	88

counterstained with van Gieson's stain; and the periodic acid-Schiff's reagent. In many instances the fixed tissue was bisected, the second portion being utilized for the demonstration of fat with scharlach R. Selected deparaffinized sections were immersed in 0.1 per cent solution of bull's testis hyaluronidase* in 0.3 per cent saline solution for 16 hours at 37° C. Serial sections from the same blocks were similarly treated in 0.3 per cent saline solution to act as controls. Other sections were incubated up to 18 hours in citrate phosphate buffer of pH 6.85, containing 0.1 mg. of ribonuclease per cc., after which all of these sections were stained with toluidine blue. It might be noted that equally good metachromatic staining was obtained with toluidine blue after routine formalin fixation as with the use of acetic acid alcohol or Herlant's fixative (95 per cent alcohol, 760 cc.; water, 40 cc.; 40 per cent formaldehyde, 90 cc.; glacial acetic acid, 45 cc.). A small group of tissues were fixed in boiling chloroform-methanol for 8 hours after which paraffin sections were prepared and stained as above. These preparations were compared with those from adjacent blocks fixed in formalin.

* The hyaluronidase in powder form was kindly supplied by John Wyeth & Brothers (Canada) Ltd., Walkerville, Ont.

Pathologic Findings

In the newborn infant the aorta was rich in elastic tissue and the endothelial cells were closely applied to the innermost elastic lamella as previously emphasized by Wilens[2] and Lansing.[5] Sections stained with toluidine blue revealed the metachromatic intercellular substance to be evenly distributed through the entire width of the wall, forming a delicate reticulated pattern interspersed between the elastic fibers. With advancing age the ground substance tended to be condensed in focal areas as prominent masses of foamy violet-tinged material readily seen with the low-power objective. If one accepts the findings in the infant as representing the normal, then it is evident that one of the early problems posed in this study was an assessment of what should be considered as normal intima. It became apparent as the investigation progressed that what is frequently considered to be normal probably represents an altered state which constitutes the earliest stage of intimal fibrosis and as such should be considered pathologic.

The most interesting feature encountered in this study was the invariable and constant accumulation of metachromatic substance in areas showing elastic fragmentation. Whenever the elastic fibers became frayed or fractured there occurred an accentuation of mucopolysaccharides at the site. With advanced elastic tissue degeneration, as seen in medionecrosis, the characteristic pooling of metachromatic substance was particularly striking (Figs. 1 and 2).

It was interesting to note that the masses of metachromatic substance did not react with the periodic acid-Schiff's reagent. However, the latter did reveal another delicate fibrillar intercellular substance which was closely applied to the elastic fibers. Sections treated with hyaluronidase showed complete inhibition of metachromasia within the wall of the aorta, while the granules of mast cells in the adventitia and near small blood vessels in the outer third of the media were unaffected. In these sections, indeed, the mast cells appeared very prominent in the absence of other metachromasia of the ground substance.

Sections stained by the Verhoeff-van Gieson method revealed intimal fibrosis, as recently described by Wilens,[2] to be present in some degree in all the adult material studied. When such a section was compared to its serial mate stained by toluidine blue a striking pattern soon became apparent. The earliest phase showed a thin zone of fibrosis situated either directly beneath the endothelium or beneath the innermost elastic lamella. Occasionally the fibrotic zone contained small fragments of elastic tissue and the lamellae lying directly beneath the fibrosis were frayed (Figs. 3 and 4). Sections stained for

fat from adjacent blocks showed distinct streaks of fine lipid droplets in the subendothelial zone (Fig. 5). Sometimes the fat extended throughout the area that was fibrosed, in other examples it was limited to short lengths. Occasionally the fat involved also the innermost portion of the media where it was intimately intermingled with the elastic fibers. In this stage the distribution of metachromatic material usually was normal, though when fragmentation or fraying of the elastic tissue was at all prominent a mild accumulation of mucopolysaccharides occurred beneath the raised endothelium (Fig. 6).

As the subendothelial zone of fibrosis became thicker, marked alterations in the inner layers of the media frequently were evident. The elastic fibers were frayed, fragmented, or completely lost (Fig. 9) either in long stretches or, at times, in focal zones extending over two or three low-power fields. In these areas a marked accumulation of mucopolysaccharides formed a distinct and often thick metachromatic band (Fig. 10). In many instances this metachromasia was not confined to the inner media but involved the thickened intima as well. In this phase it frequently was apparent that what appeared at first sight to be a very thick intima was, in reality, thickened intima plus degenerated media in which the elastica was either markedly fragmented or completely absent (Figs. 11 and 12). Fat stains in this stage always showed the presence of a moderate number of lipophages. These were more prominent in the intima but were also scattered in the area of altered media.

In a few instances when sections were taken from aortas with grossly recognizable sclerosis, the intima was found to be hyalinized. At the junction of the area of hyaline fibrosis with the media one still frequently found abnormal elastic fibers; but neither this nor the mucopolysaccharide pattern was constant. The hyalinized intima still frequently showed metachromasia of varying degrees of intensity and fat stains often showed a thick red smear of lipid in the same area. In other instances, however, the metachromatic reaction was lacking.

There was no close correlation of these changes with age. The earliest phase was seen not only in children, but also in the ascending aorta of some middle-aged and elderly patients. When sections of the ascending aorta were compared with the descending aorta of the same patient, the former frequently showed subendothelial fibrosis alone, while the latter revealed degeneration of the inner layer of media in addition to the intimal fibrosis. Medial degeneration was seen in all age groups and was particularly marked in persons with hypertension. A striking instance was observed in a female child of

11 years with de Toni-Fanconi syndrome (hereditary cystinuria). Her aorta was so involved that it closely resembled that of an elderly person (Figs. 7 and 8). Hyalinization of the intima was present only in areas of grossly visible sclerosis and was usually seen only in older adults. In 2 instances, however, it was present in the ascending aorta of females who died of acute coronary occlusion at ages 28 and 31 years respectively.

Summary of Pathologic Findings

During the development of so-called intimal fibrosis there frequently occurred progressive degeneration of the inner media in which the elastic fibers first become fragmented and then disappear. These changes may possibly be initiated by a deposit of lipid, which may only be temporary, in the intima and the inner layer of the media. A marked accumulation of metachromatic substances occurs in the area of medial degeneration and is intimately associated with fragmentation of the elastic fibers.

The Nature of the Metachromatic Substance

It has long been considered that metachromasia to toluidine blue was due to the presence of certain types of mucopolysaccharide. Since considerable work has recently been reported on these substances, an attempt has been made to identify, as closely as possible, the metachromatic substance described in these aortas.

As noted above the metachromasia was completely inhibited by hyaluronidase, while the metachromatic staining of mast cells was unaffected. This indicates that the ground substance of the aorta is probably not heparin. Since it has been stated (Wislocki, Bunting, and Dempsey[6]) that certain ribonucleoproteins may give faint metachromasia, control sections were exposed to ribonuclease, but this had no effect nor did fixation in boiling methanol-chloroform alter it. It was surprising to find that the metachromatic substance failed to stain with the periodic acid-Schiff's reagent. Leblond[7] has pointed out, however, that chondroitin sulfate does not belong to the periodic acid-Schiff's positive substances on account of substitutions in the glycol groups. Grishman,[8] in a study of mucus-producing tumors, confirmed this work and concluded that strongly metachromatic mucoid substance unstained by the periodic acid-Schiff's method was chondroitin sulfate. Recently Meyer[9] has distinguished five different mucopolysaccharides in connective tissue; namely, hyaluronic acid, chondroitin sulfate A, B, and C, and a monosulfate ester of hyaluronic acid. In his

tabulation of their occurrence in mesenchymal tissues he lists only chondroitin sulfate B and C as occurring in the aorta.

From these facts it is suggested that the metachromatic substance seen in the human aorta consists of chondroitin sulfate B and C. This is done with some misgivings, however, in view of Sylvén and Malmgren's[10] recent statement that the conditions of the metachromatic reaction do not justify its use in distinguishing between various types of acid tissue polysaccharides and further that the method of using enzymatic digestion with hyaluronidase should be regarded with skepticism until more is known about this complex group of enzymes.

Discussion

The accumulation of mucopolysaccharides in the inner media and intima is such a prominent feature in many instances of intimal fibrosis that one is forced to consider it of pathogenetic importance, but it would be unwise to attempt to draw any definite conclusion regarding its exact rôle from a purely morphologic study. One has only to read the Transactions of the Conferences on Connective Tissues[11] to realize that our knowledge of the structure and functions of ground substance is still in its infancy, though the importance of developing such knowledge is obvious when one considers that derangements of this substance may be the basic lesion in a wide variety of diseases, as recently emphasized by Boyd.[12] There are, however, several points that should be discussed briefly.

The constant association of fragmented elastic fibers and accumulation of mucopolysaccharides deserves consideration. Two possibilities are apparent: either the accumulation of metachromatic substance interferes with the nutrition of the elastic fibers so that they degenerate, or the fragmentation of the elastic fibers is primary, resulting in a pooling of the mucopolysaccharide in their neighborhood. If the former explanation is correct, one must search for factors to explain this primary increase. It seems unlikely that it represents only a redistribution of a substance normally present since birth. Morphologically, there appears to be a definite increase in the amount of mucopolysaccharides, and Faber,[13] by chemical analysis, has reported an increased carbohydrate-sulfate ester content of the aorta with age and hypertension. Gersh and Catchpole[14] have described granules, positive to the periodic acid-Schiff's reagent, within fibroblasts and suggest that these may represent a secretion product which is concerned in the production of ground substance. In the material studied here, there was no excessive fibroblastic activity to explain the increase in mucopolysaccharides, though this does not, of course, rule out the

possibility of increased secretion by the fibroblasts that were present.

On the other hand there is considerable evidence to suggest that the accumulation of mucopolysaccharides is secondary to alterations in the elastic tissue. Most authorities[15] today conceive of the formed connective tissues (reticulin, collagen, and elastic tissue) as being derived from the ground substance. Gersh and Catchpole,[14] in their detailed investigations, seem to imply that ground substance and basement membrane, at least, may be transformed from one to the other by the influence of enzyme systems altering the state of polymerization. One cannot help but wonder if such a mechanism might pertain to elastic tissue as well. Evidence to support this concept is supplied by the work of Hall, Reed, and Tunbridge,[16] who recently reported that, unlike previous observers, they found polysaccharide and sulfuric acid intimately associated with protein in elastic tissue and that polysaccharide is liberated simultaneously with protein whenever the elastic tissue is degraded. They further suggested that the enzyme elastase, which digests elastin, is not a proteolytic enzyme but rather a mucase. Baló and Banga,[17] in a brief report, noted that blood sera from man, rabbit, and cattle possess inhibitory effects upon the elastolytic activity of elastase. They also stated that elastase-inhibitor is either quantitatively decreased or absent from the blood of individuals suffering from arteriosclerosis, thus suggesting that the enzyme elastase might be active in the degeneration of elastic tissue in arteriosclerosis. However, Hall *et al.*[16] stated that the question of elasto-mucin involvement in arteriosclerosis is far more complex than that of a simple enzyme-inhibition system and suggested that more studies in the mucoid-protein interrelationship are needed in order to assess the changes involved in vascular degeneration. Wislocki *et al.*[6] also made note of the close association of elastic tissue and mucopolysaccharides, and stated that there are no exceptions to the affinity of mucopolysaccharides to elastic tissue. "Nevertheless, no real understanding of such a possible relationship will be obtained until the physical and chemical affinities of elastin and mucopolysaccharides are more fully known."

With reference to the possible rôle of the mucopolysaccharide accumulation in the production of either intimal hyalinization or focal atheroma, we are also in the field of conjecture. Moon and Rinehart[4] have suggested that hyalinization of the intima in coronary arteriosclerosis may represent an abnormal polymerization of mucoid ground substance. In the present study it was noted also that when the intima of the aorta was hyaline an intimate admixture of mucopolysaccharide and lipid often could be demonstrated. This is similar to the findings

of Moon and Rinehart in the coronary arteries and is also comparable to hyaline arteriolosclerosis. It is possible that this may represent a process analogous to the formation of the protein-mucopolysaccharide complex of amyloid.

No attempt was made to study atheromatous plaques in this investigation, but Wilens,[2] as already noted, has given reasons for assuming that intimal fibrosis is an integral part of the atherosclerotic process. It is conceivable that an accumulation of mucopolysaccharide, by altering the permeability of the subendothelial tissues, may play an important part in the localization of lipid which is the feature of the atheromatous plaque.

Another possibility has been offered by Faber.[13] He noted that cholesterol deposition is sometimes encountered in various tissues rich in metachromatic substance and suggested that the sulfate esters of the aorta might release lipid from lipoproteins brought to the tissue by the blood plasma.

Summary and Conclusions

Subendothelial or so-called intimal fibrosis is present regularly in adult aortas which grossly appeared normal or showed only minimal fatty streaking. It is also common in children.

This fibrosis probably is initiated by lipid deposition. It may be confined entirely to the intima but frequently there is an associated and profound degeneration of the inner layer of the media.

In many cases, what at first sight appeared to be intimal fibrosis proved to be subendothelial fibrosis plus degeneration of the inner media.

Medial degeneration is characterized by fragmentation and loss of elastic tissue with an associated accumulation of mucopolysaccharide, probably chondroitin sulfate.

It is suggested that the abnormally large pool of mucopolysaccharide is derived from degeneration of elastic tissue of the media and may play a part in either the development of an atheromatous plaque or in hyalinization of the aorta.

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[*Illustrations follow*]

LEGENDS FOR FIGURES

FIG. 1. Ascending aorta, female, 45 years old. Focal zone of medionecrosis showing loss of elastic fibers. Elastic tissue and van Gieson's stain. × 110.

FIG. 2. Serial section of Figure 1 showing large pool of metachromatic substance in area of medial degeneration. Toluidine blue stain. × 110.

FIG. 3. Ascending aorta with a few longitudinal fatty streaks from a male 4½ years old with subacute glomerulonephritis. There is a zone of fibrosis separating the inner elastic fibers from the underlying lamellae. Fraying of elastic fibers can be seen in the zone of fibrosis. Elastic tissue and van Gieson's stain. × 110.

FIG. 4. Male, 20 years of age. Grossly normal ascending aorta proximal to a coarctation. The fibrosis here is similar to that shown in Figure 3. Fragments of elastic tissue remain in the zone of fibrosis. Elastic tissue and van Gieson's stain. × 110.

FIG. 5. Same case as that from which Figure 3 was taken. Frozen section stained for fat. There is a streak of lipid in the subendothelial area. This corresponds to the zone of fibrosis shown in Figure 3. Sudan IV stain. × 110.

FIG. 6. Same case as that from which Figure 4 was taken. Bubbling clumps of mucopolysaccharide lie beneath the undulating surface. Fat also was present here. The distribution of metachromasia in the media is the normal adult pattern. Toluidine blue stain. × 150.

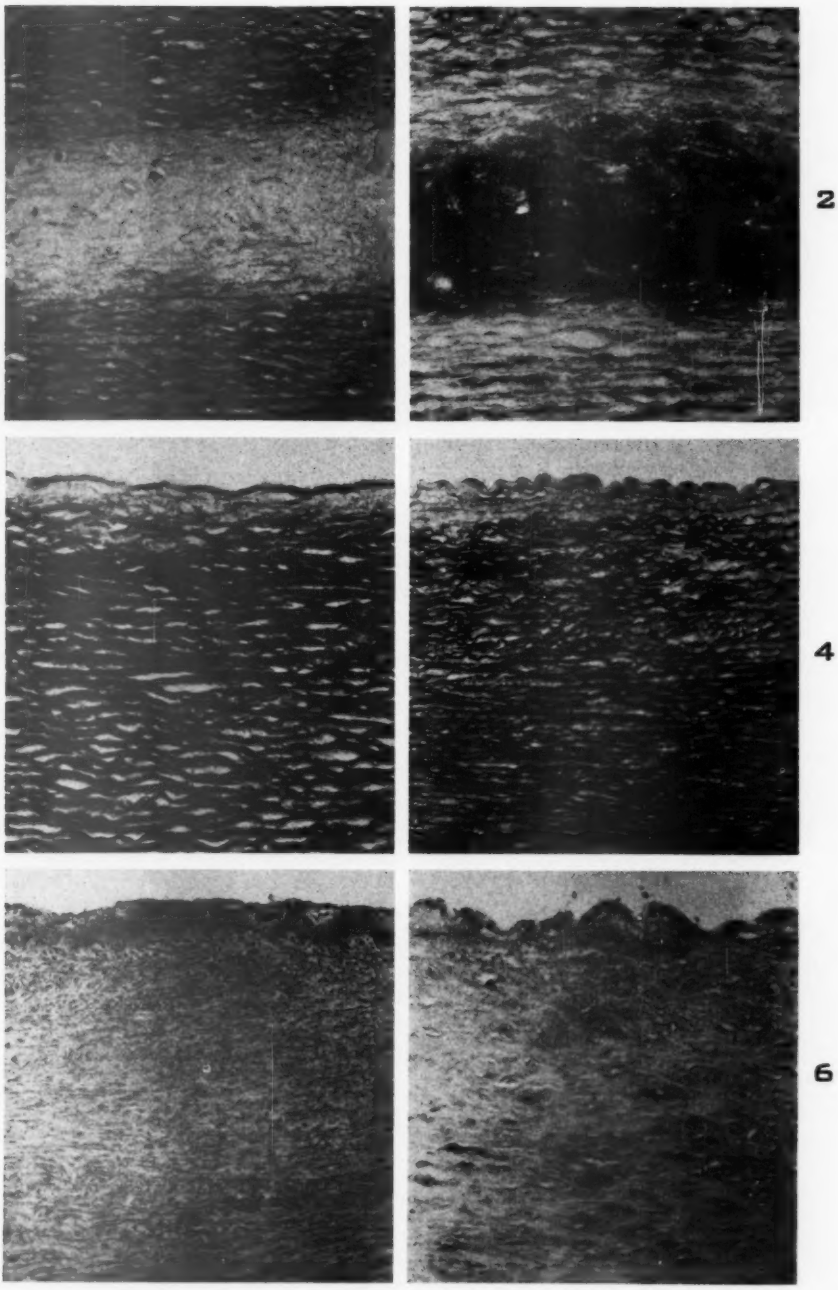
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FIG. 7. Thoracic descending aorta, grossly normal, from a female, 11 years old, with hereditary cystinuria. At the top there is a thin subendothelial zone of fibrosis (A). Beneath this the inner media shows complete loss of elastica (B). The elastic fibers of the remaining media (C) are widely separated from each other and fragmented. Elastic tissue and van Gieson's stain. × 110.

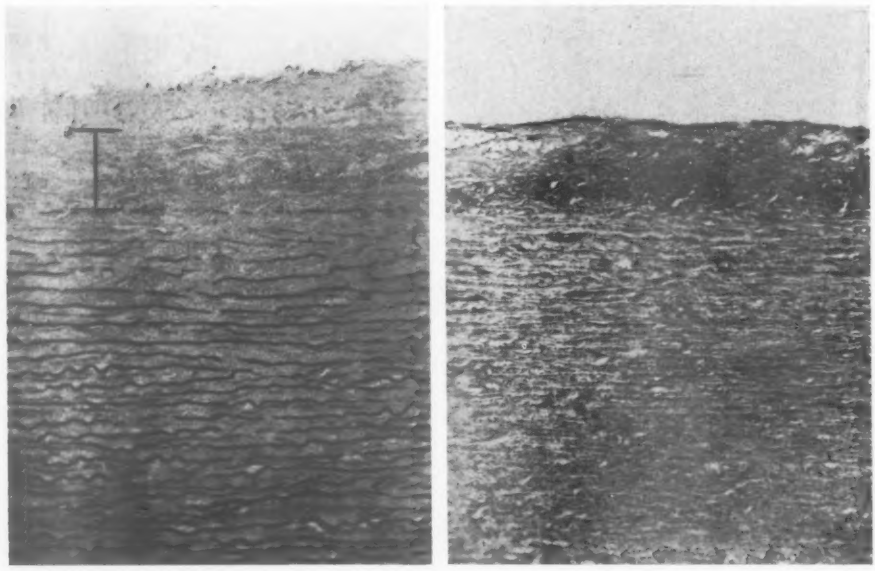
FIG. 8. Same case as that from which Figure 7 was taken. This section is from the same ribbon but there is artefactual loss of the subendothelial fibrotic zone. The deep band of metachromasia corresponding to the degenerated media (B) in Figure 7 is quite apparent. Of note also is the general increase in metachromatic material through the entire wall. Toluidine blue stain. × 110.

FIG. 9. Ascending aorta, which showed grossly a few fine, fatty streaks; male, 46 years of age, with hypertension. There is a thin subendothelial zone of fibrosis at the top. Beneath this can be seen a typical example of marked elastic tissue fragmentation in the inner media. The remainder of the media is normal. Elastic tissue and van Gieson's stain. × 110.

FIG. 10. Same case as that from which Figure 9 was taken. Section from the same ribbon as Figure 9 to show wide zone of deep metachromasia corresponding to the zone of elastic tissue degeneration seen in Figure 9. Toluidine blue stain. × 110.

FIG. 11. Ascending aorta containing a few fine, longitudinal, fatty and gray streaks, from a male, 35 years old, with malignant hepatoma and thrombosis of the portal vein. At the top the subendothelial zone is thickened. Beneath this can be seen a wide band of media from which the elastic tissue has disappeared except for a few small fragments. The remainder of the media is normal. Elastic tissue and van Gieson's stain. × 110.

FIG. 12. Serial section from the same case as that from which Figure 11 was taken. The broad band of metachromatic substance corresponds to the zone of medial degeneration in Figure 11. Toluidine blue stain. × 110.



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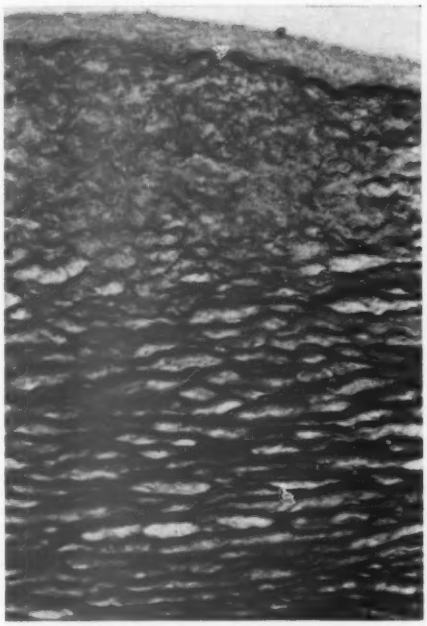

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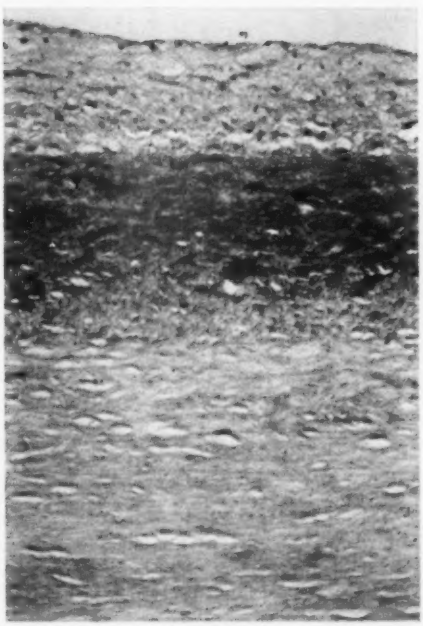

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THE MUSCULATURE OF THE LUNGS IN CHRONIC PULMONARY DISEASE *

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Numerous fragmentary and several extensive systematic accounts of hyperplasia and hypertrophy of the pulmonary musculature in disease have been published. These publications are not well known. It now appears desirable to prepare a summary of available information, to add new observations, and to attempt an assessment of the mechanisms involved in muscular hypertrophy and hyperplasia and of the functional significance of these processes as they affect various structures in the lung.

Distribution of Muscle in the Normal Lung

Macklin,[1] in his review of 1929, stated: "The fact that the lungs are muscular organs, in a very real sense, has become increasingly obvious in recent years." Early knowledge of the musculature of the air passages was contributed by Reisseisen[2] who performed painstaking dissections to the level of the bronchioles of the muscle now appropriately bearing his name. The most detailed modern study has been published by Baltisberger[3] who based his description upon the histologic study of the lungs of a single individual, an executed criminal, 25 years of age. Subsequent observers, such as Lénárt,[4] von Hayek,[5] and ourselves, have come to the conclusion that either the lungs of this man were most remarkable for their large content of muscle or that they were, in fact, not normal. Indeed, Figures 14 and 15 in Baltisberger's publication suggest that this subject may have had a degree of pulmonary emphysema. Nevertheless, although it may be disputed in some details, his minute description will be employed as a basis of discussion.

Baltisberger[3] conceived of the musculature of the lung as consisting of two systems, the first continuous with the muscle of Reisseisen and the second situated within the interstitial tissue. Moleschott,[6] in his

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thesis (1845), described muscle fibers in the walls of the alveoli, but it is now generally agreed that the former, or bronchial system, extends no further than the walls of the last orders of alveolar ducts. About the openings of the first subdivisions beyond (infundibula, in the usage of Husten[7] and of Baltisberger[3]; atria in the sense of Miller[8,9]), the muscle fibers exist in interlacing bundles in a more or less sphincteric arrangement. These most distal muscular bands appear as "knobs" in cross section or as bands when met in other planes. They are more prominent in some species, such as the guinea-pig, than in man. Miller[9] has described the arrangement of these fibers as "geodesic," that is applied to the surfaces of the curved structures in such a way as to exert, when under tension, an inward force in the direction of the radius of the curvature. There has been disagreement concerning the existence of muscle in the interstitial tissue, including that of the pleura and septa. Von Hayek[5] has denied that there is normally any muscle in the pleura of man, and Miller[9] stated that he could find this tissue only in the pleura of the guinea-pig. Baltisberger, however, in his subject, demonstrated smooth muscle in association with the pleural and septal lymphatics, not only as a component of the walls of these structures, but also as pursuing a longitudinal course more or less in parallel to them. He also found muscle fibers in the connective tissue about certain of the veins.

Muscle in Pulmonary Disease: Previous Reports

The existence of muscular hypertrophy and hyperplasia in the lung under pathologic circumstances was recognized as early as 1872, by Rindfleisch[10] in a study of "brown induration." Arnold[11] had reported a remarkable hyperplasia of muscle in the pleura even earlier (1867) and had quoted the thesis of Leo-Wolf[12] that had been published in 1832 but without the benefit of modern histologic techniques. Orth[13] soon confirmed Rindfleisch's observation on brown induration and Eberth[14] found a striking increase of muscle tissue in the lungs of cats suffering from caseous pneumonitis. The term muscular cirrhosis was applied by Buhl[15] and has subsequently been used by others (Davidsohn,[16] Tanaka,[17] and von Stössel[18]) for conditions in which proliferation of muscle fibers and connective tissue in the lung are combined.

In such material, proliferated muscle has been described not only in the walls of bronchioles (Eberth,[14] Davidsohn,[16] and Fossel[19]) but

more peripherally in the interalveolar septa (Tanaka,[17] Bowden,[20] and Roubier *et al.*[21]), and "interstitially" in carnified lungs (Blumauer,[22] Harkavy[23]). Several instances of muscular proliferation in "honeycomb" lungs (von Stössel,[18] Rosendal,[24] Oswald and Parkinson[25]) and in the walls of congenital cysts (Berg and Vejlens[26]) have been described. Rosendal presented an instance in which the proliferation involved not only the walls of the air passages but also those of lymphatics both within the pulmonary parenchyma and in the regional lymph nodes. An association with, but not necessarily a derivation from blood vessels has been mentioned by Lénárt,[4] Davidsohn,[16] Fossel,[19] Dejerine and Sottas,[27] and Buchmann,[28] among others. Several have also considered the possibility of the new development of muscle from primitive connective tissue elements but have preferred in the end to think of the proliferated muscle as derived from preexisting muscle cells.

It is clear that, although valuable observations have been made, much still remains to be learned concerning the origin, distribution, and functional import of the muscular tissue in pulmonary disease.

Material and Methods

Material for the present study was gathered simply by setting aside for detailed consideration histologic sections of lungs that appeared to contain an unusual amount of muscle. These slides were encountered during the routine examination of necropsy material and of pulmonary tissue resected surgically. An idea of the conditions under which large quantities of muscle may be observed is obtainable from Table I. Here are listed the basic final diagnoses in all material (436 specimens) submitted from the thoracic surgical service during a period of approximately 4 years, together with all cases in these various categories that came under the present special study. This tabulation is not intended to give more than an inkling of the minimal incidence (in this series approximately 4.6 per cent) of notable hyperplasia and hypertrophy of muscle in chronic disease of the lung. No attempt was made at a quantitative assay and nothing more than the judgment of the observers dictated the selection of material. Moreover, the stated diagnoses indicate merely the major condition. Associated with the latter, there was almost always pneumonitis in various stages of organization, often the remarkable vascular changes that accompany chronic pulmonary disease, and sometimes pulmonary emphysema. During the

same interval of 4 years additional specimens* were collected, chiefly from necropsies. The selected material, a total of 40 lungs or lobes (Table I, column 3), was restudied in a systematic fashion, preparatory to the present report.

In general, muscle tissue could be identified beyond doubt in the hematoxylin and eosin preparations; the Masson and van Gieson stains in many instances yielded little additional information. The muscle cells were more plump than the fibroblasts, more sharply outlined, more intensely acidophilic, and often vacuolated. Their nuclei were larger and ovoid or rod-like, with rounded ends rather than elongated, and spindle shaped, in contrast with the nuclei of fibroblasts. They

TABLE I

Distribution of Total Material, of Lungs with Notable Muscular Hypertrophy or Hyperplasia, and of Material Selected for Study

Primary diagnosis	1. Total surgical lung specimens (approx. 4 yrs.)	2. Specimens with over-growth of muscle (from column 1)	3. Total specimens for present study*
Emphysema	12	5	13
Bronchiectasis	87	8	13
Abscess	37	5	7
Bronchitis and bronchiolitis (including asthma)	9		4
Tuberculosis	119	1	2
Tumor	132	1	1
Other	40		
Total	436	20	40

* Includes material listed in column 2.

took hematoxylin more deeply but retained a reticular structure. The muscle fibers usually were arranged in bundles interlacing with others or lying isolated within collagenous material. Further identification was based on the resemblance of the cells in question to smooth muscle in its usual position, as in the walls of bronchi or vessels, within the same preparation. Often the proliferated fibers not only resembled, but appeared to be indisputably in continuity with, the musculature of

* Dr. Paul Kimmelstiel of Charlotte, N.C., and Dr. Robert Fienberg of Boston, Mass., each graciously contributed a valuable specimen and gave permission to include it in the present study.

these structures. In some preparations there was evidence of a gradual collagenous replacement of muscle. The myoid origin of these foci could still be distinguished by virtue of the persistence of some fibers and by the similarity of their fascicular arrangement to that of unhyalinized groups of muscle cells. In association with the collagenous replacement, the muscle fibers had become atrophic.

Observations

The descriptions will be based on the predominant location of the muscle tissue. As in various earlier observations, proliferated muscle was found (1) in association with the air passages, (2) in association with blood vessels, (3) in association with lymphatics, (4) in apparent isolation within the connective tissue of the pleura and septa. The muscle in these various locations will be described in turn, although frequently there was evidence that it had been derived from several sources. In some lungs, however, large masses of muscle were present whose origin could not be established with certainty. Of special interest was the abundant muscle found in certain instances of bullous emphysema and these specimens will receive separate discussion.

Muscle Associated with the Respiratory Passages

For the most part the greatest contribution to the increased musculature could be related to the walls of the air passages, beginning with the larger bronchi. Here the muscle often was present in a strikingly thick layer, and groups of fibers parallel to those of the bronchus extended into the pulmonary substance beyond, pushing aside or often enveloping the smaller air passages (Figs. 1 to 4). The myoid tissue sometimes was arranged in bundles, related not concentrically but spirally or even longitudinally to the bronchus, or in an eccentric fashion. Muscular hyperplasia frequently was especially prominent about the bronchioles so that a small lumen lined by cuboidal epithelium became embedded in an immense mass of large fibers, clearly separated from the surrounding tissue (Fig. 3). It is interesting to note that as serial sections were examined at a distance from the level illustrated in Figure 3, no epithelium-lined spaces could be seen. Such tangential sections are to be expected, since the layer of muscle may be very large in relation to the size of the lumen. Thus, serial sections are necessary to determine the relationships of apparently isolated masses of myoid tissue in the parenchyma.

About more distal air passages, not obviously enveloped in the manner described, there could be seen also large bundles or sheets of muscle. In this position muscle has often been spoken of as interstitial. This peripheral distribution is common in chronic passive congestion, as has been described by several observers beginning with Eberth,[14] but it is also frequently encountered in the organizing pneumonitis associated with bronchiectasis, lung abscess, tuberculosis, and other conditions (Figs. 5 to 8). The relation of this tissue to the air spaces would suggest its continuity with the muscle of the respiratory bronchioles and alveolar ducts, but since in the material under study these passages are often devoid of epithelial lining, they may represent either alveoli or more proximal parts of the respiratory tree. The frequently large size of the spaces suggests the latter (Fig. 8), but there appears to be no compelling reason why the myoid tissue in the course of its proliferation cannot penetrate more distally. With the obliteration of the air spaces, muscle is often left embedded in granulation tissue (Figs. 5 and 6).

In chronic bronchitis, and especially in bronchiectasis, the mucous membrane of the bronchi frequently is found to have a trabeculated appearance. In part, this is associated with proliferation of muscle fibers within folds of mucous membrane (Fig. 9). Hyaline transformation can then take place as within muscle elsewhere (Fig. 10). On occasion a polypoid mass consisting largely of such hyalinized muscle may be formed (Fig. 11), and this may be of sufficient size in itself to obstruct the lumen of a major bronchus (Fig. 11).

Large quantities of muscle tissue could be identified within fibrotic pulmonary parenchyma even upon gross examination, by virtue of its tan-pink color, translucency, and resiliency, which contrasted with the white, more opaque, and less yielding fibrous material.

Muscle Related to the Blood Vessels

In chronic disease of the lung, striking changes may take place in the pulmonary blood vessels. Thrombosis, ultimately with recanalization and shrinkage, is commonly observed in the pulmonary arteries and veins. On the other hand, the bronchial collateral circulation—arterial or venous, or both—may be remarkably expanded. In the bronchial arteries there develops a very prominent inner longitudinal muscular coat that ultimately exceeds in thickness the outer circular layer. Accompanying this muscular proliferation, there may be thickening of the elastic lamina but on occasion the latter is interrupted.

Prominent circular bands of elastica can appear on both sides of the longitudinal muscle but usually it is thicker on the luminal side, suggesting to some that the longitudinal muscle is a part of the media, rather than of the adventitia. Such bronchial vessels often have been mistaken for segments of the pulmonary circulation with "degenerative changes." The prominent longitudinal muscle is typical of the bronchial arterial system, but it can be observed also in arteries clearly identifiable as pulmonary rather than bronchial.

Vessels with a prominent inner longitudinal muscular coat have been described in the normal lung, especially by von Hayek,[29] Merkel,[30] and others who have considered them as "Sperrarterien," that is, arteries capable of making or interrupting blood shunts between bronchial and pulmonary arteries and even veins, and subserving a putative function of the lung as a "depot" or storage mechanism for blood. Many writings dealing with this subject are speculative and attempt to draw conclusions regarding function merely from anatomical observations and without physiologic confirmation. Whatever their status in the normal lung, greatly enlarged bronchial vessels are prominent in chronic pulmonary disease and especially in bronchiectasis in which their anastomoses with the pulmonary arteries (but not pulmonary veins) can be demonstrated easily (Wood and Miller,[31] Liebow, Hales, Harrison, Bloomer, and Lindskog,[32] Lapp[33]). Despite the large size which their lumina may possess, their walls are often disproportionately thick. Encroachment upon the lumen may continue, reducing it to a mere slit (Figs. 12 to 16). In some vessels there can be seen smaller subsidiary channels, likewise with muscular walls, that have the appearance of canalizing vessels, but these are not penetrating granulation tissue but among the longitudinal muscle bundles (Fig. 12). Sometimes there is complete obliteration of the lumen by masses of myoid cells in longitudinal arrangement (Fig. 16).

It is interesting to note that similar obliterative changes consequent to proliferation of longitudinally arranged muscle fibers can take place in branches of the intercostal arteries as they traverse the pleura through adhesions to become a part of the collateral supply (Fig. 16). The existence of these transpleural vessels, which are obviously newly formed, casts doubt on the presumed functional significance of the structure of the "Sperrarterien." The obliterative process consequent to muscular hyperplasia is a remarkable method of reducing a collateral circulation that does not subserve a very useful function in chronic pulmonary disease, but which may on the contrary constitute a burden

upon the heart, especially the left side. When the walls of such vessels are met tangentially in section, they may be mistaken for free lying muscle bundles. More careful observation will, however, disclose the outer circular coat and, usually, at least remnants of the elastic laminae.

In many specimens, there was evidence that a further contribution to the musculature of the diseased lung had stemmed from the walls of these vessels. This consisted of an apparent splitting off of bundles of muscle with subsequent proliferation. The arrangement is spiral, in a manner recalling the whorls of a nebula. The outer bundles, although still centered upon the arteries, become commingled with fascicles of muscle cells in continuity with the proliferated bronchial musculature (Figs. 14 and 15). In certain fields, it may no longer be possible to name with certainty the source of the fibers.

Muscle Associated with Lymphatics

Considerable masses of muscle may be associated with proliferated or dilated lymphatics in the lung. It is little realized that extensive lymphatic proliferation may occasionally be a feature of chronic pulmonary disease. Rosendal[24] has described such changes occurring with a peculiar microcystic transformation of the respiratory passages. The lymphatics may occur predominantly in the pleura and especially in the septa and extend thence into the parenchymal organization tissue (Figs. 17 to 19). The proliferated myoid fibers can be a part of the actual thickened wall of the lymphatic, or the fibers can form an irregularly thick, longitudinal investment, or they can merely accompany the channel but remain at some distance. In that case they can exist apparently free in the areolar connective tissue stroma. Why these proliferated muscle fibers should be associated with the lymphatics is unknown. They occur, analogously, within and about the lymphatics as they enter the regional nodes (Fig. 20). This is not a finding peculiar to diseased lung, but occurs normally in lesser measure. Moreover, the musculature of the lymphatics in other tissues can under certain circumstances become greatly hypertrophied and proliferated, as for example in carcinoma of the breast (Fig. 21).

Muscle in the Pleura and Septa

Remarkably thick sheets of smooth muscle fibers can be observed in the pleura and septa in association with the lymphatic proliferation that has been discussed. They can also be found when there is pulmo-

nary emphysema, as will be described (Fig. 24), and occasionally when there is an organizing process in the parenchyma.

"Free" Muscle Tissue

Certain bundles of muscle situated in the pulmonary substance are not clearly associated with the walls of respiratory passages, blood vessels, or lymphatics. Serial sections are necessary to establish the fact. The probable origin of such masses of muscle is from the walls of bronchioles, or more distal air passages whose lumina have been obliterated by granulation tissue, with subsequent shrinkage. In the connective tissue of the pleura and septa, proliferated muscle may be derived from fibers, that, according to Baltisberger,[3] normally lie free within these membranes.

Muscle in Pulmonary Emphysema

The existence of an abundance of muscle in the lung in certain instances of pulmonary emphysema of the so-called atrophic, senile, or bullous type was noted in a brief paper by Lénárt[4] published in 1923. As mentioned by him, excessive muscle is not found in all examples of emphysema. In the present observations it was found in abundance in 5 of 12 specimens when surgical resection was performed with the primary intent of removing emphysematous tissue (Table I).

When present, myoid tissue is found not only in the walls of the bullae, but may also be remarkably abundant within the air passages leading into them (Figs. 22 to 28). The latter resemble the bronchioles as seen in asthma, but are often of smaller size and usually lack a thick basement membrane and a heavy leukocytic, especially eosinophilic, infiltration. In emphysema, entering bronchioles not only may possess very thick muscular coats, sometimes partly hyalinized, but the actual channels of communication with the zones of emphysematous change as traced in serial sections may be exceedingly narrow (Fig. 22). In the walls of the bullae themselves can be traced myoid tissue clearly derived from all of the various sources identified in other types of chronic pulmonary disease, with the possible exception of lymphatics. It seems reasonable to suppose that most of the muscle tissue in the minor septa among the smaller vesicles is derived from the distal air passages, such as alveolar ducts (Figs. 22 to 24). Striking is the contribution from the blood vessels whose immensely thick walls are replete with bundles of longitudinally arranged muscle fibers (Figs. 27 and 28). All stages of obliteration of the lumina of these vessels

can be observed. The origin of the muscle that may thicken the pleura is not clear, unless it is derived from "free" fibers within that membrane (Fig. 24).

Discussion

Doubtless an understanding of the pathogenesis of pulmonary emphysema would illuminate the problem of the mechanisms involved in the development of the often concurrent muscular hyperplasia, and of its functional significance, but this knowledge is still far from complete and the theories are numerous and diverse.[34]

It is often said that senile or bullous emphysema is the consequence of overstretching of alveoli resulting from loss of elasticity. Measurements indicating an increase of intrapleural pressure in this condition leave no doubt that the total pulmonary substance exerts a decreased elastic traction upon the visceral pleura. This does not, however, mean that decrease in elastica is the primary pathogenetic mechanism. Objective studies such as those of Orsós[35] do not necessarily substantiate the commonly held belief that loss of elastic tissue *precedes* the expansion of the air spaces. Both Orsós[35] and Sudsuki[36] have described continuous subtle destruction of pulmonary substance in emphysema beginning with the walls of expanding alveoli. The latter become increasingly thin and lacunae of increasing size develop in the interalveolar septa. Orsós has considered these to be the result of tears, while Sudsuki has regarded them as enlarged pores of Cohn. Elastica can still be distinguished in the attenuated walls, although in the form of fibrils more delicate than usual. It is possible that entrapment of air resulting from such muscular hyperplasia of bronchioles as has been described is responsible for over-distention of alveoli and consequent transformation of the elastic tissue. Other changes in bronchioles leading to fibrosis, narrowing, and increased rigidity, with a similar interpretation in regard to the pathogenesis of associated emphysema, have been described by Amberson and Spain[37] and by Heppleston.[38] As the bulla becomes a space-occupying lesion, the surrounding structures, including bronchi, are displaced, stretched, and narrowed upon its more or less spherical surface, with a tendency toward further obstruction—resulting in a "vicious cycle."

With the further progress of the destructive process in emphysema, adjacent air spaces of increasing size come into communication one with another, and bullae are formed. These often can be shown to communicate with several bronchioles or even bronchi, even though a degree of valve-like obstruction exists. In other instances the connections

with bronchi up to several millimeters in diameter are free and direct. Communications among adjacent air spaces and the multiple inosculations of bronchi with the bullae have been demonstrated clearly with the casting technique by Loeschcke,[39,40] and their existence has been confirmed by similar methods in our laboratory. It is thus clear that a bulla represents far more than an over-expanded alveolus, but is rather the residue of a considerable mass of pulmonary substance. The remnants of the walls of bronchioles and their accompanying vessels persist for a time as trabeculae within the bulla, but subsequently collapse upon the outer wall of the latter, contributing their contents of residual and often proliferated muscle. During this process, vessels become obliterated, and this, in many instances, is accompanied by remarkable muscular proliferation, as has been demonstrated. At the same time, a rich collateral blood supply, chiefly venous, is built up, a process that again involves proliferation of myoid tissue.[41,42] Moreover, emphysema often is accompanied by organizing pneumonitis that may add to the muscular content, as has been described.

The abundance of muscular tissue within bronchioles leading into the emphysematous portions of certain lungs may help to explain the beneficial effects of bronchodilator drugs in some patients. Further study of this muscle as a target tissue in allergic reactions, and in its responses to chemical and biologic agents, is needed.

The fundamental factors concerned in the muscular hypertrophy and hyperplasia found in fibrosing pulmonary disease are still obscure. In some respects the process may represent the response of smooth muscle subjected to increased tissue tensions, perhaps analogous to the hypertrophic changes taking place in the arrectores pilorum in dermatomyositis. Most mysterious are the causes of muscular proliferation in the vessels, especially that of the longitudinal layer in the greatly enlarged bronchial arteries as seen especially in bronchiectasis.

Summary and Conclusions

In many types of chronic pulmonary disease, there occurs a remarkable hypertrophy and hyperplasia of muscle. Much of this muscle is derived from several identifiable sources: (1) bronchi and more distal air spaces, (2) blood vessels, especially the longitudinal layer of the bronchial arteries, (3) lymphatics, (4) interstitial tissue not clearly associated with other structures. Muscle fibers from these sources may become commingled.

In vessels, the lumen may become completely obliterated as a con-

sequence of muscular hyperplasia, thus abolishing a useless or even burdensome collateral circulation.

Hyperplasia of muscle is especially striking in some instances of pulmonary emphysema, in which the walls of the bullae contain masses of myoid tissue derived from all of the sources mentioned previously. Compaction of the residual tissue, occurring concurrently with the destructive process responsible for the development of the bullae, in part accounts for the abundance of muscle in the walls. The bronchioles leading to the emphysematous region may possess great collars of muscle and narrow lumina, perhaps producing or contributing an obstructive factor to the development of emphysema. Relaxation of this muscle by certain drugs may explain relief of symptoms in certain patients with this condition, but further study of its responses is required.

The fundamental mechanisms responsible for the muscular hypertrophy and hyperplasia are not fully understood. One element may be the effect of entrapment of these fibers at increased tensions, especially in tissue undergoing fibrosis.

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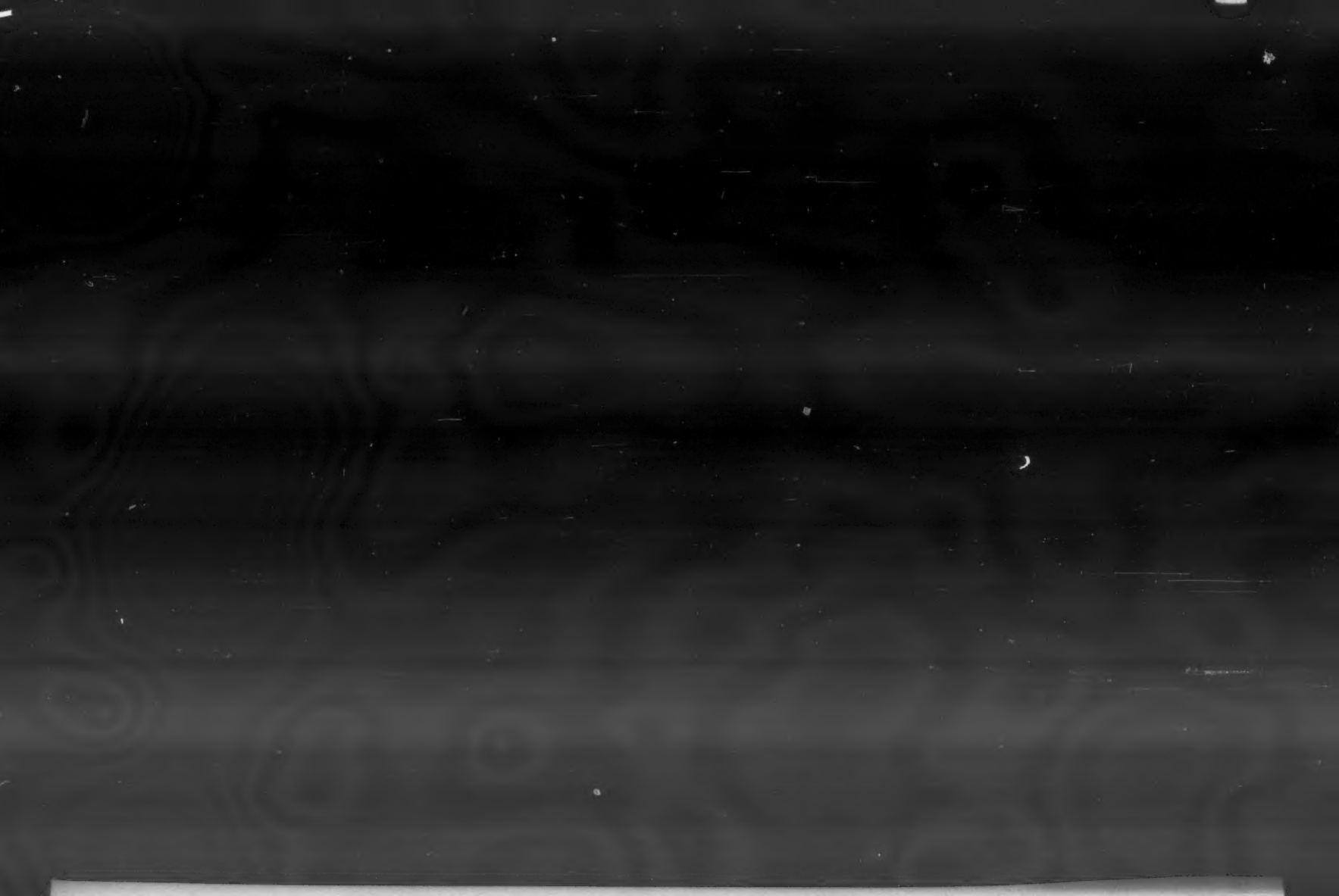
LEGENDS FOR FIGURES

FIG. 1.* Numerous fine fasciculi of muscle fibers forming a remarkably thick coat in the wall of a bronchiectatic sac. × 155.

FIG. 2.* Thick-walled arteries amidst muscle bundles from the same specimen that is illustrated in Figure 1. × 180.

FIG. 3. A minute bronchiole lined by cuboidal epithelium surrounded by an enormously thick muscle layer. In another section the lumen was not seen. The patient was a 64-year-old woman with episodes of pneumonitis in 1916 and 1946 and residual pulmonary abscess in the superior segment of the lower lobe, together with bronchiectasis in the basal segments. Resection performed in 1951. × 180.

* The material for Figures 1 and 2 was kindly supplied by Dr. Paul Kimmelstiel.



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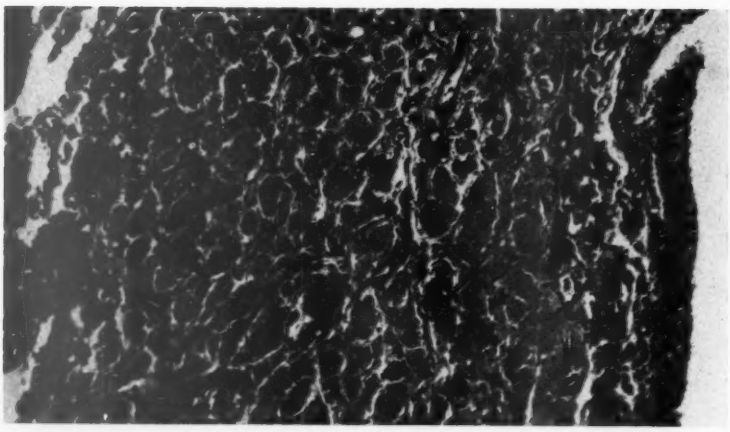

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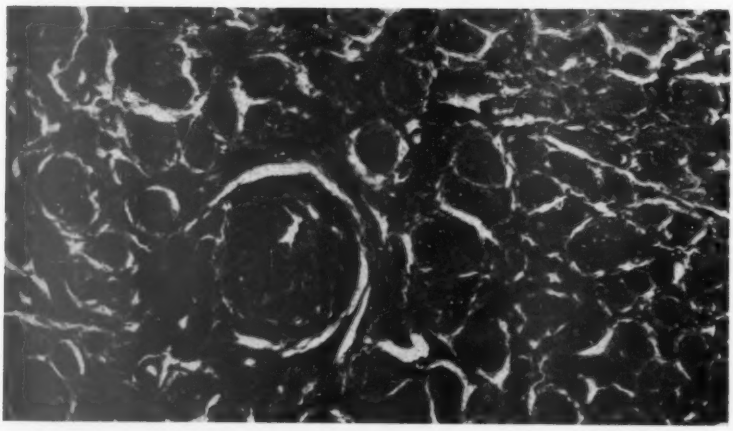

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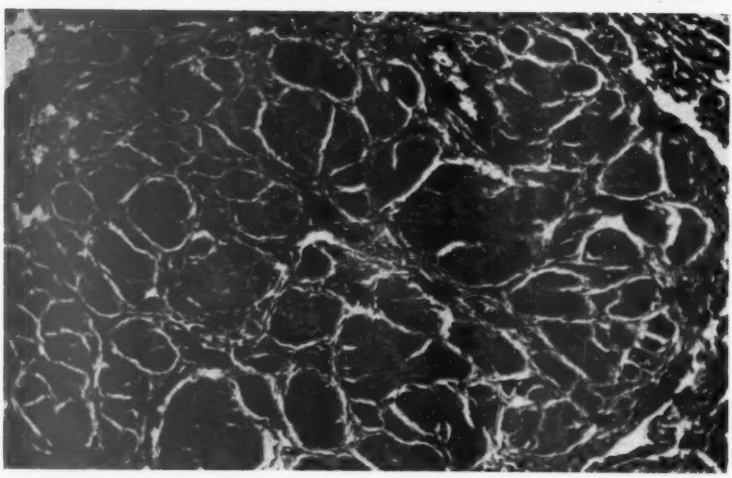

FIG. 4. Parallel bundles of muscle fibers arranged concentrically about a bronchiectatic cavity, but enclosing altered air spaces of the surrounding parenchyma. These air spaces are lined by low cuboidal, or flattened epithelial cells and probably represent the residua of respiratory bronchioles or of alveolar ducts. From the lung of a 56-year-old woman who had pneumonitis following a cholecystectomy in 1947. The left lung was the seat of bronchiectasis and was resected surgically in 1951. × 120.

FIG. 5. Another section (from the lung illustrated in Fig. 4) with almost complete obliteration of residual air spaces within granulation tissue containing prominent groups of elongated muscle fibers. × 120.

FIG. 6. Another field (from the lung illustrated in Figs. 4 and 5) with apparent "interstitial" position of the muscle bundles. This probably results from obliteration of all air spaces by granulation tissue. × 120.

FIG. 7. A mass of muscle associated with a pigmented scar in the lung of a man, 57 years old, with asthma of long duration. The lungs showed evidence of emphysema, as well as numerous small scars. × 75.

FIG. 8. Bundles of "interstitial" muscle in the walls of alveolar ducts in a lung which is the seat of passive congestion. The patient, a 57-year-old man, had severe mitral stenosis with decompensation of at least 9 years' duration and right cardiac failure. × 120.

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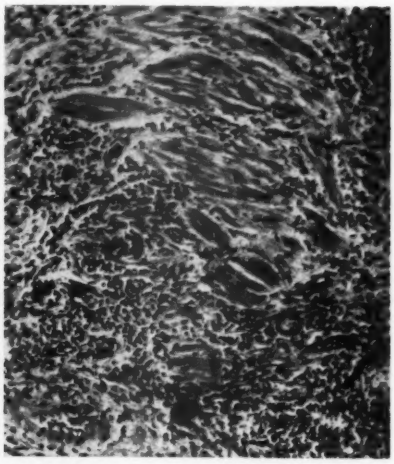

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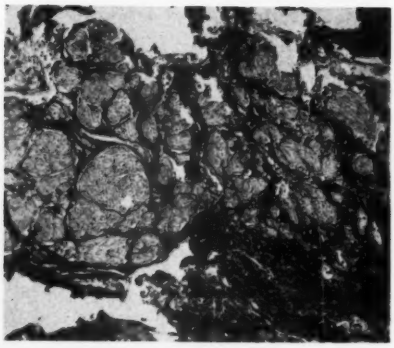

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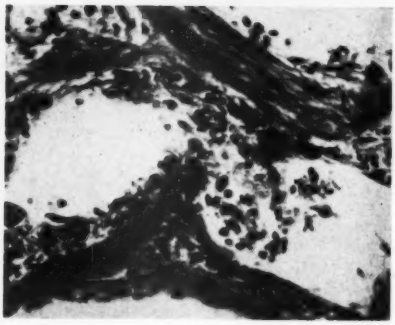
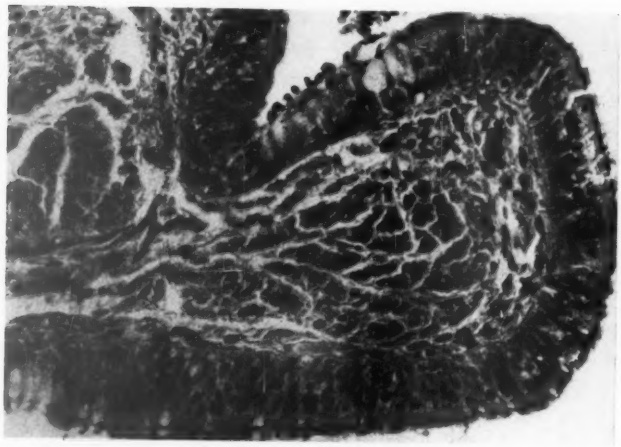


FIG. 9. Trabeculation in mucous membrane of a bronchus from a bronchiectatic lung. The hypertrophied and hyperplastic muscle tissue forms the core of a prominent ridge. The patient was a 57-year-old woman whose symptoms of chronic cough and episodes of hemoptysis began at the age of 25 following pneumonia. There was bronchiectasis of all lobes of the resected left lung and a polypoid mass obstructing the posterior segmental bronchus (Fig. 11). × 175.

FIG. 10. Hyaline change within muscle tissue deeper in the wall of a bronchus (from elsewhere in the lung illustrated in Fig. 9). A few myoid fibers persist within collagen that has largely replaced the bundles of muscle, whose outlines can still be discerned. × 120.

FIG. 11. View of polypoid mass partly occluding the posterior segmental bronchus of the lower lobe (another section from the lung illustrated in Figs. 9 and 10). Much of the substance of the polyp consists of hyalinized masses of muscle similar to that illustrated in Figure 10. × 19.



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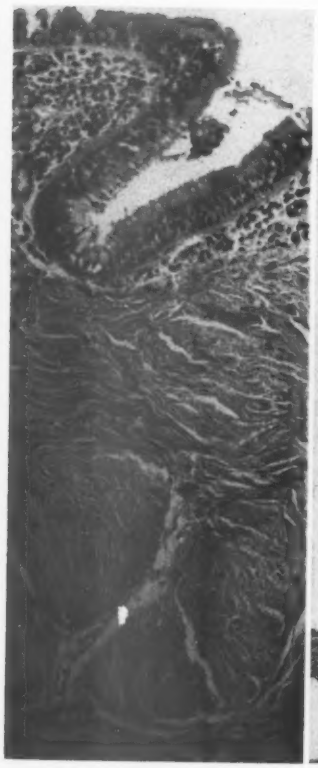

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FIG. 12. Bronchial artery with remarkable proliferation of longitudinal muscle fibers. Two slit-like lumina are seen, giving somewhat the appearance of branching; so many of these channels are seen, however, that the process suggests more an unusual form of canalization in a vessel in process of obliteration by muscular proliferation. From the lung of a 62-year-old man with a history of productive cough following influenza in 1918. The lower lobe resected in March, 1951, was the seat of bronchiectasis. × 175.

FIG. 13. Another bronchial artery from elsewhere in the lung illustrated in Figure 12. There are numerous isolated bundles of longitudinal fibers and the lumen is eccentric. × 120.

FIG. 14. A bronchial artery in the wall of a bronchiectatic sac is seen to be surrounded by whorls of muscle bundles, chiefly longitudinal, some of which are continuous with those of the bronchus (from elsewhere in the lung illustrated in Figs. 12 and 13). × 120.

FIG. 15. A bronchial artery from another instance of bronchiectasis. The patient was a 58-year-old man who had had pneumonia in infancy, influenza in 1918, and "bronchial asthma" for 25 years. Bronchiectasis demonstrated roentgenographically. Death in 1951 following complications of pyelonephritis and amyloidosis. × 120.

FIG. 16. Branches of intercostal arteries in the parietal pleura traversing adhesions. The changes in these vessels are similar to those in the intrapulmonary branches of the systemic arteries as seen in fibrosing disease. Various stages in the obliterative process are demonstrated. The lumen of the largest vessel has disappeared and the resemblance to a nerve is striking. There is, however, a thin outer circular layer and a massive core of longitudinal muscle fibers. From elsewhere in the lung illustrated in Figure 15. For comparison with Figures 28 and 29. × 95.

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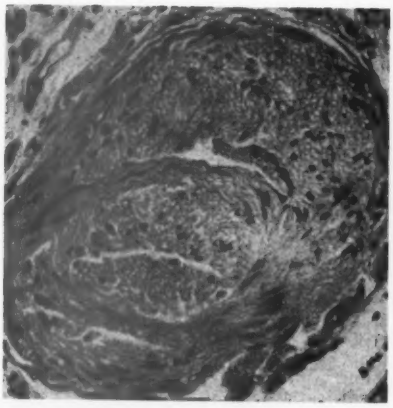

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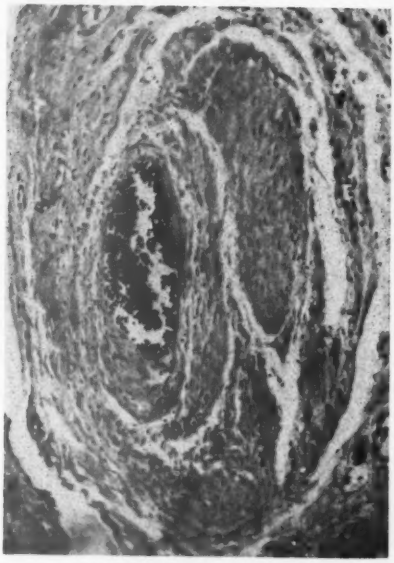

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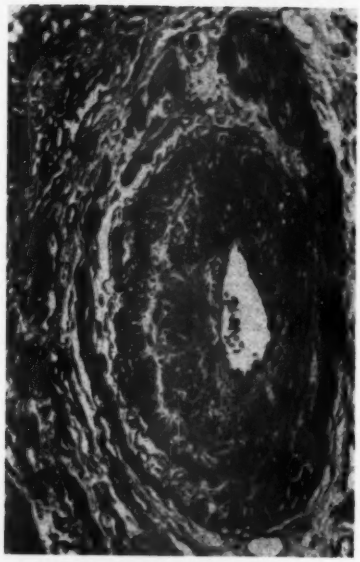

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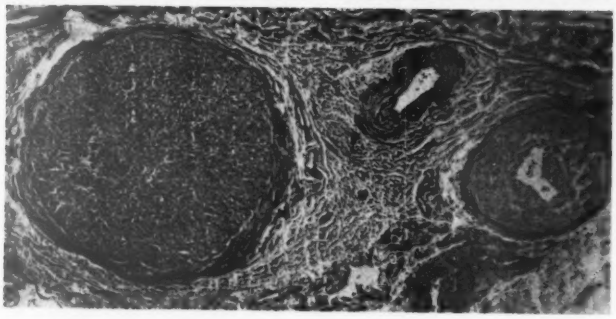

FIG. 17. Pleural lymphatic in an emphysematous lung from a 77-year-old man. The muscle coat has become greatly and irregularly thickened. The muscle fibers, as in the arteries, are chiefly longitudinal. The valves of the lymphatic are seen. Finely granular material occupies the lumen. × 120.

FIG. 18. Numerous distended lymphatics within and outside of a septum in organizing pneumonitis. The lymphatics are accompanied by chiefly longitudinal bundles of smooth muscle fibers, but some of the latter lie apparently free in the loose connective tissue. The patient was a man, 22 years of age, who had necrotizing pneumonitis 1 year previously, with residual abscesses in the left lower lobe. × 62.

FIG. 19. Higher magnification of one of the lymphatics seen near the lower border of Figure 18. × 150.

FIG. 20. Smooth muscle in the capsule of a lymph node and in the wall of an entering lymphatic. × 180.

FIG. 21. Hyperplasia of longitudinal muscle with obliteration of the lymphatics in a breast, the seat of carcinoma. A fragment of tumor can be seen within a small channel eccentrically related to the large mass of muscle. × 120.

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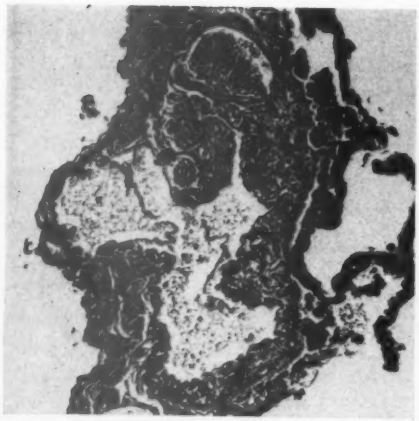
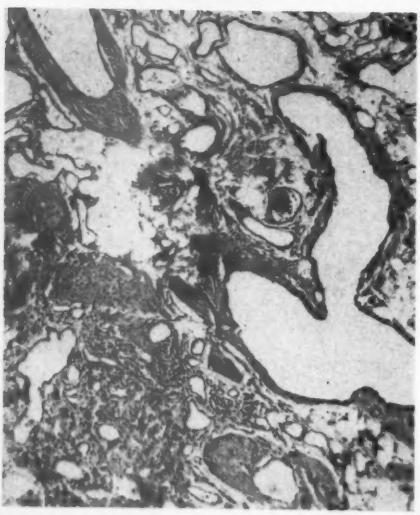
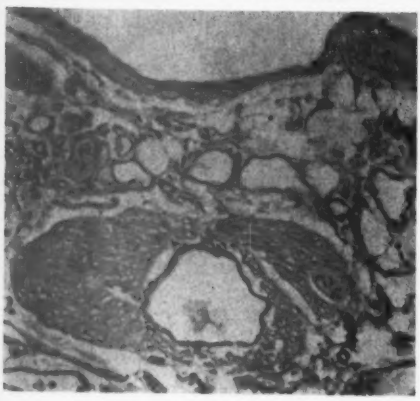
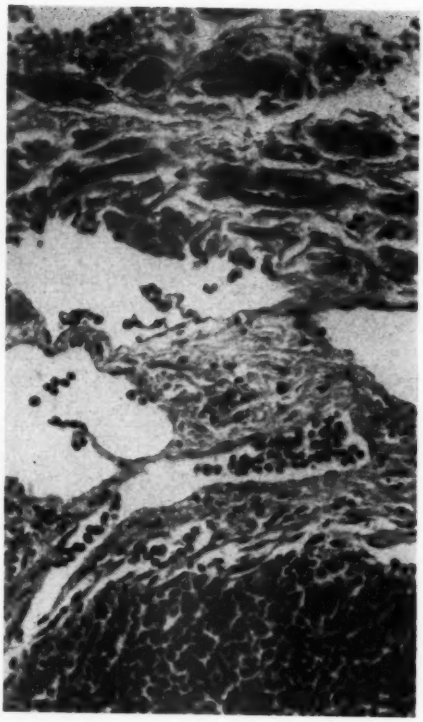








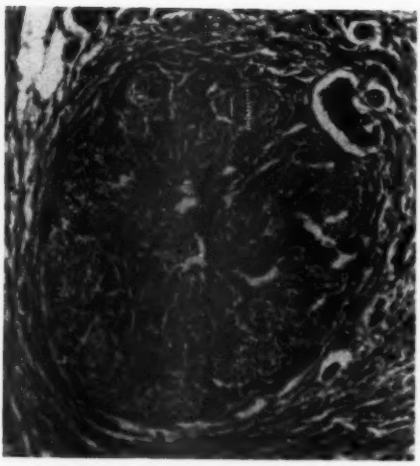
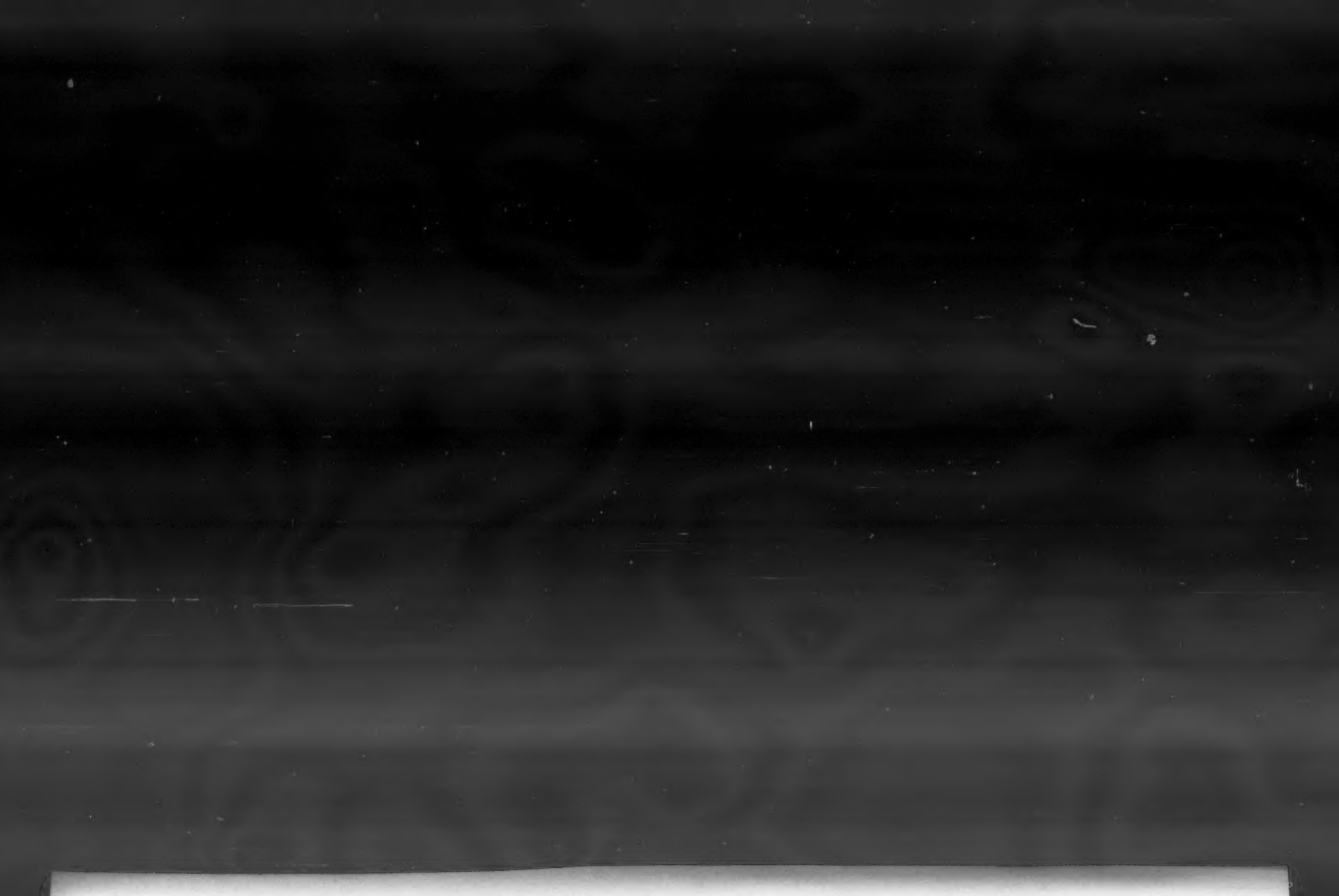


FIG. 22. Tortuous and narrow bronchioles with prominent muscle bundles at the entrance to a localized region of subpleural emphysema. Peripheral portions of the emphysematous tissue are shown in Figures 23 to 25. The patient was a 41-year-old man in whom a focus of emphysematous change was encountered and excised incidentally in the course of a transthoracic vagotomy for relief of peptic ulcer. × 120.

FIG. 23. Mass of muscle tissue partly surrounding a small bronchiole, again near the entrance to the emphysematous labyrinth, whose trabeculae likewise contain thick muscle bundles. × 120.

FIG. 24. Trabeculae within emphysematous bullae replete with muscle, some sectioned transversely and some longitudinally. The multiple intercommunications among the air spaces are obvious. × 120.

FIG. 25. Prominent muscle bundles in the pleura. × 120.



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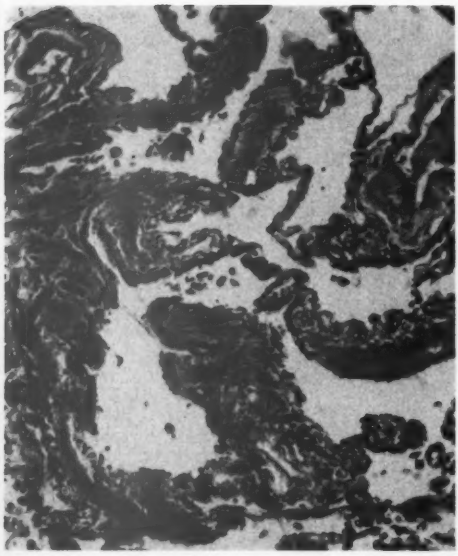

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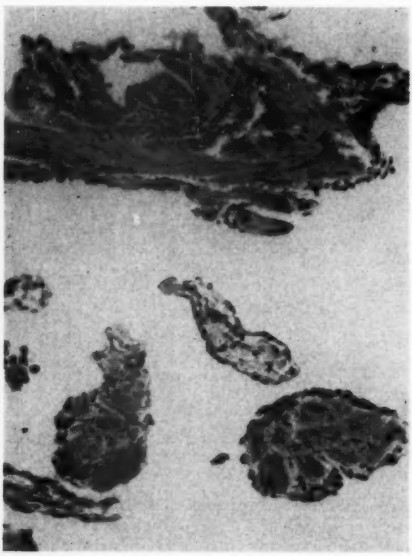

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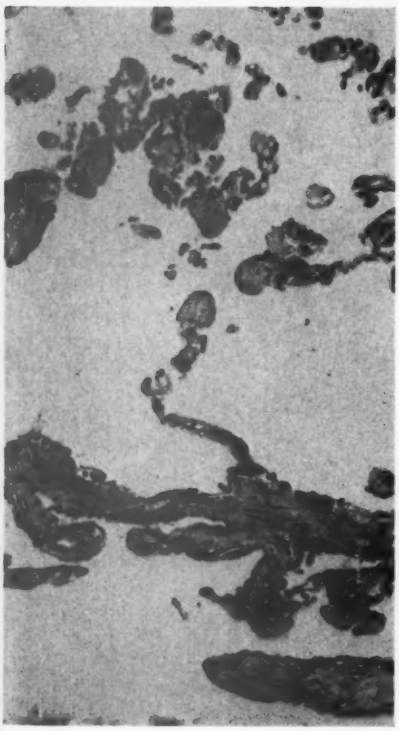

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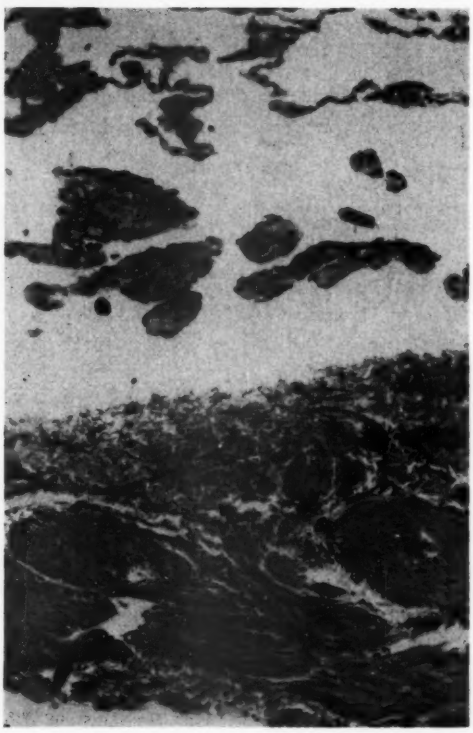

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FIG. 26. The walls of emphysematous sacs from the periphery of a lung, the seat of bullous change. The bulk of each interbullous septum consists of muscle tissue that has become hyalinized in a few places only. From a 54-year-old man with a 5-year history of shortness of breath. Spontaneous pneumothorax occurred just before operation for excision of the bullae. × 35.

FIG. 27. A higher magnification of a portion of Figure 26, displaying the vacuolated muscle cells. The lining of the bulla consists of a thin layer of granulation tissue. × 170.

FIG. 28. Residual vessels in pulmonary substance undergoing bullous emphysematous transformation. A number of vessels are seen, each sectioned almost transversely, with varying degrees of obliteration of the lumen by proliferated muscle fibers. From a 50-year-old man whose right lung was excised for carcinoma. The emphysema was an incidental finding. × 30.

FIG. 29. The uppermost of the vessels seen in Figure 28, under higher magnification. The fact that the wall consists almost entirely of longitudinal muscle fibers is demonstrated. A minute lumen is present but it does not contain red blood cells. × 120.

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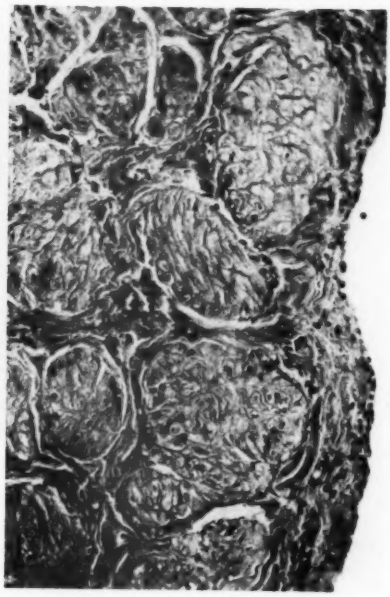

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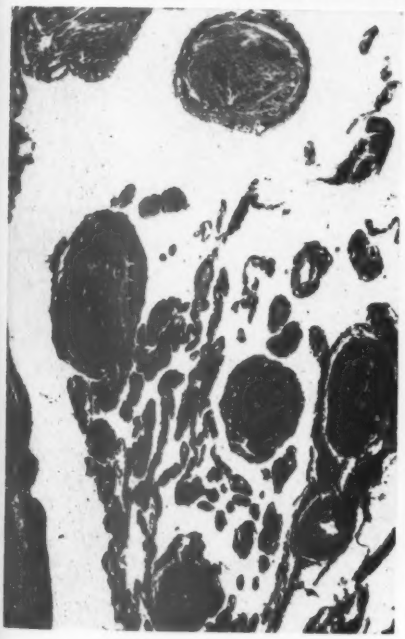

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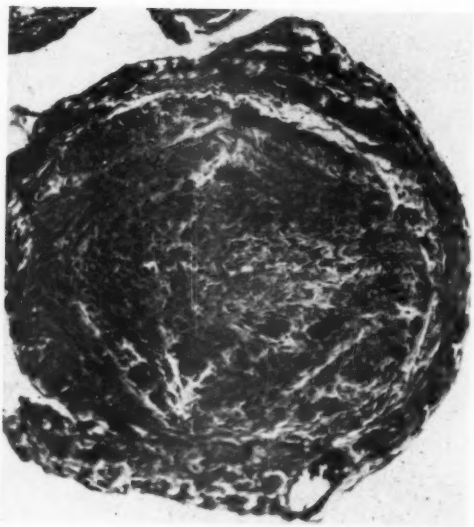

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NECROTIZING GRANULOMATOSIS AND ANGIITIS OF THE LUNGS AND ITS RELATIONSHIP TO CHRONIC PNEUMONITIS OF THE CHOLESTEROL TYPE *

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In a previous publication,[1] two cases of necrotizing granulomatosis and angiitis of the lungs with massive splenic necrosis and focal, thrombotic, granulomatous glomerulonephritis were described. The lesions found in the lungs of these cases were of special interest since they resembled in many aspects certain lesions of the lungs which have been called chronic pneumonitis of the cholesterol type by Robbins and Sniffen[2] and Waddell, Sniffen, and Sweet,[3] and foam cell pneumonia by Chiari.[4] This resemblance led to the study and comparison of seven pulmonary lesions that had been removed surgically and had been placed in the category of atypical pneumonitis. One of the cases with marked vascular involvement and focal necrosis will be considered separately as a case of idiopneumonic necrotizing granulomatosis and angiitis; the other six, which possessed the features of chronic pneumonitis of the cholesterol type and were similar histologically, will be discussed as the combined group. The term idiopneumonic is applied to the first of the seven surgically removed pulmonary lesions in order to differentiate it from cases of necrotizing granulomatosis and angiitis in which the lesions of the lungs are accompanied by involvement of other organs and to which the term disseminated necrotizing granulomatosis is applied.

IDIOPNEUMONIC NECROTIZING GRANULOMATOSIS AND ANGIITIS

A 37-year-old white male bench worker in a gun factory was first admitted to this hospital on June 12, 1947, with the complaint of having coughed up a cupful of bright red blood 10 days previously. In 1943, a sudden gush of bright red blood from the mouth had occurred. Following this episode, he was hospitalized at two Army Hospitals and discharged in August, 1944. Since that time, he had had sharp, non-radiating pain in the right chest. In 1945, he took sulfonamide pills for 2 weeks following a tooth extraction. In 1947, he spent 1 month in a hospital for tuberculosis, having experienced night sweats, moderate shortness of breath, wheezing, coughing with

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small amounts of sputum, fatigue, occasional hemoptysis, and chest pains, but sputum examinations for acid-fast bacilli and tuberculin tests were negative.

From the time of the first admission to this hospital in June, 1947, until September, 1950, when the patient was admitted for the seventh time, he continued to have the same complaints, which became progressively more severe. The temperature rose to 99.8° F. Repeated physical examinations revealed coarse inspiratory and expiratory rhonchi, sibilant and sonorous râles, and wheezing in the right chest. Breath sounds were diminished over the right upper chest anteriorly and posteriorly. The systolic blood pressure varied from 110 to 130 mm. of Hg and the diastolic blood pressure from 75 to 90 mm. of Hg. The vital capacity was 3 liters. Urinalyses were negative. The hematologic findings were within normal limits, the white blood cell count varying from 6,400 to 9,450 and the eosinophils from 1 to 5 per cent. The sedimentation rate was 11 mm. (Wintrobe). Alpha streptococci, *Staphylococcus aureus*, *Neisseria catarrhalis*, but no acid-fast bacilli were found in the sputum. A tuberculin test with second strength purified protein derivative (tuberculin) was positive. A roentgenogram on the first admission disclosed a fairly well demarcated, triangular patch of consolidation measuring 1.0 by 4.0 cm. in the basilar portion of the right upper lobe, limited below by the horizontal fissure which was slightly elevated. There was also a rounded but less well demarcated patch of consolidation approximately 3.0 cm. in diameter in the anterior basal bronchopulmonary segment of the right lower lobe. A right calcified paratracheal lymph node and several smaller discrete areas of calcification were noted in the hilar regions. This roentgenologic picture did not vary in the subsequent admissions. Bronchoscopic examinations were negative. A right multiple segmental lobectomy was performed on October 2, 1950. Three masses were removed, one in the anterior segment of the right upper lobe and two in the inferior segment of the right lower lobe. Dense vascular adhesions were found between the chest wall and the masses. Following the operation, the patient continued to have shortness of breath and wheezing. Asthma appeared in February, 1951, and became severe enough to necessitate hospitalization. He was last seen in December, 1951, still suffering from asthma.

Surgical Specimen

Gross Examination. Three pieces of lung were submitted, weighing together 168 gm. The largest contained a portion of the anterior margin of the upper lobe and measured 11.5 by 9.0 by 4.0 cm. The other two pieces were from the lower lobe, one measuring 6.5 by 4.0 by 0.2 cm. and the other 3.0 by 2.5 by 2.5 cm. In each piece a subpleural, well circumscribed, firm, compact, wedge-shaped mass was present, measuring 2.0 to 3.0 cm. in diameter. Each mass was well demarcated from, and surrounded by, varying amounts of pulmonary parenchyma which was non-crepitant, non-fibrotic, meaty, and grayish black, with scanty margins of spongy parenchyma more peripherally. The masses cut with difficulty, and the sectioned surfaces were black to grayish white with small cavities occasionally seen containing granular, reddish gray linings and measuring 0.3 to 0.8 cm. in diameter. A segmental bronchus infrequently entered a mass but did not appear to communicate with the cavities. The bronchioles and bronchi near the masses were, at times, dilated and had thick walls. The

pleura was slightly ragged, dark reddish purple, with some whitening occasionally over the masses.

Bacteriologic Examination. Tissue from the masses was macerated and injected into 4 guinea-pigs. One died at the end of 13 days of bronchopneumonia, and the others were sacrificed at the end of 8 weeks and were found to be normal.

Microscopic Examination. The tissues were fixed in Zenker's acetic acid fluid and stained with hematoxylin and eosin, phosphotungstic acid hematoxylin, Mallory's aniline blue-orange G stain, Masson's trichrome stain, Verhoeff's elastic tissue stain, Foot's modification of Hortega's silver carbonate stain for reticulum, Gram's stain, Giemsa's stain, the stain for acid-fast bacteria, and the periodic acid-Schiff's stain. Formalin-fixed tissue was used for identifying anisotropic crystals, for the Sudan IV stain, and for the Schultz stain for cholesterol.

Multiple sections of the three nodules disclosed similar findings. Large regions of dense acellular fibrous tissue with broad bands of collagen and no elastic fibers were prominent. Scattered plasma cells and lymphocytes were found in the fibrous tissue. Where the pleura was in contact with the nodules, it had become incorporated into the fibrous tissue. Occasionally, the collagenous fibrous tissue had undergone coagulative necrosis with the bundles of collagen still recognizable and nuclear débris among them. In the aniline blue-orange G stain, these necrotic bundles of collagen often stained red, suggesting acidophilic homogenization (fibrinoid degeneration). Occasionally, a small mass of necrotic collagen was surrounded by a row of palisaded cells among which a few foreign body giant cells were seen. A further stage in the process of necrosis was the loss of tingibility of the collagen bundles, best observed in the aniline blue-orange G stain, and the formation of foci of granular disintegration in which neither collagen bundles nor reticulum fibers could be recognized. Cholesterol crystals were present. The collagen bundles forming the walls of the necrotic foci ended abruptly at the margins of the areas of necrosis where occasionally palisaded fibroblasts could be found. No inflammatory cellular infiltrate was directly related to these necrotic foci. Very rarely, a small group of cells resembling epithelioid cells, with an infrequent multinucleated giant cell, was found near a necrotic focus.

Many of the bronchioles and small bronchi, particularly in the lower lobe, were dilated and lined by metaplastic stratified squamous epithelium. Ulceration and necrosis of the walls of some of the bronchioles and small bronchi produced cavities lined in part by necrotic granula-

tion tissue containing numerous vacuolated macrophages, in part by metaplastic squamous epithelium, and in part by acellular fibrous tissue. Some cavities were lined only by necrotic granulation tissue. Masses of neutrophils and granular débris were found in the lumina of the cavities and recent hemorrhages were present in the walls. About both the non-ulcerated and ulcerated bronchioles, a cellular fibroblastic tissue, rich in reticulum fibers, was found. This contained scattered neutrophils, lymphocytes, eosinophils, plasma cells, a few vacuolated macrophages, and multinucleated giant cells with either peripherally arranged or scattered nuclei. This cellular fibroblastic tissue was sharply demarcated from the more peripheral, acellular, collagenous fibrous tissue. In the larger bronchi, particularly those near the hilus, squamous metaplasia was present but no ulceration. Arteries and veins throughout the nodules possessed markedly thickened, edematous, fibrous intimal layers often infiltrated with plasma cells, lymphocytes, and eosinophils.

Some blood vessels were completely occluded by organized, recanalized thrombi. The walls of the blood vessels often were disorganized and fibrotic, and occasionally thickened by marked reduplication of the elastic laminae. Extensive fragmentation and disappearance of the elastic laminae were seen in both arteries and veins. Some blood vessels could be recognized only by the presence of fragments of the elastic laminae. In arteries and veins which passed from the fibrotic tissue into the adjacent negative lung parenchyma, the segments in the fibrotic tissue possessed a thickened intima and a fibrotic media and adventitia with almost complete loss of the elastic laminae, while the directly joined segments in the adjacent pulmonary parenchyma were entirely negative. At the edges of the dense fibrotic tissue, alveoli were being surrounded and invaded by an extension of the fibrous tissue. The walls of these alveoli were thickened by fibrosis, increased numbers of reticulum fibers, and an infiltrate composed of lymphocytes, eosinophils, neutrophils, and plasma cells. Masses of vacuolated mononucleated and multinucleated macrophages were present within the lumina and walls of these alveoli. There was, at times, a sharp demarcation between the nodules and the adjacent uninvolved pulmonary parenchyma. Occasional peribronchiolar and perivascular lymphocytic infiltration with follicle formation was noted. In tissue stained with Sudan IV, positively stained lipid droplets were found in the masses of macrophages within the alveolar walls and lumina, in the macrophages within the necrotic granulation tissue lining the cavities, and in the fibroblasts of the fibrous tissue. Similar lipid material

permeated the necrotic bundles of collagen. In the Schultz stain, positive staining material was found in the lipid. Anisotropic droplets, many in the form of Maltese crosses, were found in the same regions as the Schultz-positive droplets. Gram's, Giemsa's, periodic acid-Schiff's, and acid-fast stains of tissue sections failed to disclose any organisms.

COMBINED GROUP

PNEUMONITIS OF THE CHOLESTEROL TYPE

The other six pulmonary lesions removed surgically are considered as a combined group because of the similarity of the histopathologic picture. All patients were males from 40 to 63 years of age. The duration of symptoms was from 2 weeks to 1 year. In 3 cases, the illness began with symptoms of an upper respiratory infection and, occasionally, these symptoms became recurrent. Cough, chills, fever, sweating, anorexia, fatigue, malaise, loss of weight, elevated temperature, slight to moderate hemoptysis, dyspnea, dull to sharp chest pain, in some instances aggravated by coughing or breathing, and gray, brown, or yellow, foul sputum were present. In one case, chest pain occurred insidiously and a cough which had been present for several years had persisted unchanged. Sudden dyspnea with sharp pleuritic pain and fever to 104° F. were present in another case. No history of asthma, hay fever, or urticaria could be obtained from any of these patients except for the fifth, who had had asthma as a child. A sulfonamide had been administered to one patient 4 years before the onset of pulmonary symptoms; the others gave no history of such therapy. The following physical signs were found over the affected portion of the lung: diminished expansion, resonance and breath sounds, increased dulness, fremitus, friction rub, fine and coarse râles, and bronchial breathing. Clubbing of fingers and toes was found in 2 cases and a cyanotic tinge of fingers and toes in one case. Blood pressures varied from 110 to 160 mm. of Hg systolic, and 70 to 80 diastolic. Results of urinalyses were negative except for one case with a trace of albumin and 0 to 1 red blood cells. The white blood cell count varied from 6,150 to 21,000. In one case, the eosinophils ranged from 2 to 6 per cent with a white blood cell count of 9,000. This patient had received a sulfonamide. The Kahn serologic test was negative in all cases. No acid-fast bacilli were found in the sputum using various methods, including guinea-pig inoculation. An alpha streptococcus and a coagulase negative *Staphylococcus aureus* were isolated from the sputa. Numerous smears of sputa were negative for neoplastic cells. In 4 cases, a skin test was positive to purified protein derivative (tuberculin).

In one case, roentgenograms revealed a collapsed right upper lobe with consolidation and a small central radiolucency about 1.0 cm. in diameter. A mass in the upper portion of the right hilar region was seen also. In the second case, patchy consolidated foci in the apical segments with small cavities and slight elevation of the left main bronchus were observed. A roentgenogram of the chest taken a year previously was negative. In the third case, there were extensive patchy consolidation, nodulation, cavitation, and marked contraction of the upper half of the right lung. Considerable elevation of the right hilum and suggestive involvement of the apical segment of the lower lobe and middle lobe were seen also. In the fourth case, there was parenchymal infiltration of the right upper lobe with central rarefaction and a fluid level. Roentgenograms of the chest of the fourth case taken 4 and 2 years previously were negative. In the fifth case, there was spotty consolidation of the entire left lung with several small cavities suggested in the upper lung fields. In the sixth case, a large region of consolidation with a wedge-like apex leading to the right hilum was noted in the lower portion of the right upper lobe near the periphery. Bronchoscopic

examinations disclosed no evidence of obstruction. Clinical diagnoses of abscess, chronic suppurative process, carcinoma, tuberculosis, and bronchiectasis usually were made. As for treatment, in 2 cases a right upper lobectomy was done; in a third, a left pneumonectomy; in a fourth, a right pneumonectomy; in a fifth, right upper and middle lobectomies; and in a sixth, a left pneumonectomy. All the patients did well postoperatively but the follow-up periods were too short for comment.

Surgical Specimens

Gross Examination. The lesions consisted of wedge-shaped or rectangular, indurated, rubbery, non-crepitant subpleural masses. At times, a large part of a lobe was affected. The pleura often was incorporated in the mass, and the pleural surface was either smooth or ragged. The masses occurred either singly or as multiple masses in different lobes. The greatest number of masses in any lung was three. The masses possessed diameters of from 2.0 to 8.0 cm. There was no predilection for any particular lobe or for any part of a lobe. Occasionally the masses were well demarcated from the adjacent uninvolved pulmonary parenchyma. On section, the tissue usually was compact and black with prominent yellowish white speckling. Occasionally, white peribronchial and perivascular fibrous columns separated parenchyma containing reddish yellow flecks. The walls of the bronchioles and small bronchi were thickened and the lumina dilated forming cyst-like structures measuring up to 1.5 cm. No obstruction was found in the larger bronchi. Some of the bronchioles contained yellowish-green mucoid material. Hilar lymph nodes were, at times, slightly enlarged but usually were soft, black, and small.

Bacteriologic Studies. Smears and cultures of macerated tissues of one specimen for acid-fast bacilli were negative as was a guinea-pig inoculation. Studies carried out in this case by the Special Bacteriology Laboratories, Communicable Disease Center, Public Health Service, Chamblee, Georgia, revealed a *Corynebacterium acnes;* Bacteroides species undetermined, as it grew very poorly in pure culture; and an anaerobic streptococcus. In another case, tissue was taken from the left upper and lower lobes. Smears and cultures for acid-fast bacilli were negative. A smear stained with Gram's stain was negative, but routine aerobic and anaerobic cultures yielded an alpha streptococcus. A fungus culture was negative. Two guinea-pigs inoculated subcutaneously with a tissue suspension were found to be negative at the end of 6 weeks.

Microscopic Examination. The tissues were fixed and stained as were those of the separately considered case of idiopneumonic necrotizing granulomatosis and angiitis. The last 6 cases possessed a similar

histopathologic picture, so that a composite description will serve. In the more involved regions, edematous, loose, acellular connective tissue enclosed small alveolar remnants. Occasional patches of more cellular fibrous tissue were seen also, with regions of acellular dense fibrous tissue with broad bands of collagen. Within this fibrous tissue, abundant coarse, short, wavy elastic fibers were noted. Abundant reticulum fibers were found also. Scattered lymphocytes, plasma cells, and vacuolated macrophages were present in the fibrous tissue with a few lymphoid follicles. Sheets of lymphocytes surrounded alveolar remnants in some areas. Eosinophils were fairly numerous, particularly in the more cellular fibrous tissue. Here and there, multinucleated giant cells of the foreign body type with anthracotic granules were scattered in the fibrous tissue. Some of the foreign body giant cells were about cholesterol crystals, others were phagocytizing elastic fibers. Groups of alveoli surrounded by the fibrous tissue had walls thickened by elastic fibers and reticulum or by a cellular infiltrate composed of lymphocytes, plasma cells, macrophages, and eosinophils. Many of the alveolar walls were edematous. Infrequently, the thickened alveolar walls were collagenized. The lining alveolar cells often were swollen, and much desquamation was taking place. Infrequently, a group of neutrophils was found within the alveoli. Masses of vacuolated macrophages, some with intracytoplasmic anthracotic granules, were in the alveolar lumina. Other vacuolated macrophages had migrated into the alveolar walls causing marked thickening. Occasionally, plugs of organized exudate were seen in the alveolar lumina.

The bronchioles and small bronchi were dilated, forming the cavities seen grossly. Some had intact epithelium; others were completely ulcerated; and still others were partly lined by either columnar or metaplastic stratified squamous epithelium. Granulation tissue in which numerous swollen fibroblasts were present was found below the ulcer. This granulation tissue sometimes was covered by a thin layer of fibrin threads and neutrophils, and contained plasma cells, lymphocytes, neutrophils, macrophages, and multinucleated giant cells of the foreign body type. Compact cellular fibrous tissue was found distal to the granulation tissue with more cellular fibrous tissue further to the periphery. The lumina of some bronchioles were completely occluded by granulation tissue and others were filled with neutrophils or vacuolated macrophages. The walls of the bronchioles and small bronchi with intact epithelium were infiltrated with plasma cells, eosinophils, and lymphocytes. In the peri-mural tissues of both ulcerated and non-ulcerated bronchioles and small bronchi, plasma cells and

eosinophils were found. In some, the basement membrane was thickened and hyalinized. In less involved regions, peribronchiolar and perivascular fibrous tissue was abundant and extended as wide septa peripherally to enclose groups of well preserved alveoli. Within this fibrous tissue and the walls of adjacent alveoli, scattered plasma cells, lymphocytes, and eosinophils were seen. Further to the periphery, unaffected pulmonary parenchyma was found except for intra-alveolar red cells and precipitated protein. Some of the involved regions were separated from negative parenchyma by a dense fibrous band.

The walls of many of the arteries and veins in the involved regions, including the fibrous tissue septa, were altered. Arteries and veins were found with thickened, fibrous, edematous, intimal layers, often infiltrated by plasma cells and lymphocytes. Occasionally, collagen was found in the thickened intimal layers, replacing the abundant reticulum. In some arteries and veins the lumina were greatly narrowed. Others were completely occluded by organized, recanalized thrombi infiltrated occasionally by plasma cells and rare eosinophils. The rest of the wall was thickened by reduplicated elastic fibers which had often replaced the media, occasionally in an eccentric manner. Small amounts of collagen sometimes were seen in the media. In one vein, phagocytosis of the elastic fibers by multinucleated foreign body giant cells was observed. The arteries and veins within the normal parenchyma were negative. No lymphatic obstruction was seen.

The large bronchi were negative except for an occasional peribronchial focus of calcification and areas of mucosal metaplastic squamous epithelium. In the Sudan IV stain, numerous macrophages filled with lipid droplets were seen in the fibrous tissue, in the walls and lumina of the alveoli, and in the granulation tissue lining the bronchi and bronchioles. Many of the lipids were anisotropic and, after heating, Maltese crosses were observed. The Schultz stain revealed positive-staining lipid in many of the macrophages. No acid-fast bacilli were found. In 3 cases, rare to few Gram-positive cocci and bacilli were found in the superficial portion of the granulation tissue lining the small bronchi and bronchioles or in the fibrin on the surface of the granulation tissue. Similar organisms were seen in the inflammatory exudate within the lumina. In one case, infrequent masses of branched filaments with peripheral clubbing were seen in the lumina of dilated, ulcerated bronchioles. No organisms were found in one case. The pleura over the involved portions was thickened by fibrosis and incorporated with the fibrous pulmonary parenchyma. Adjacent to the

broad, fibrous patches in the parenchyma, acellular fibrous septa extended from the pleura into the parenchyma or else a wedge-shaped scar containing anthracotic pigment extended down from the pleura. The hilar lymph nodes were not remarkable except for anthracosis and enlarged germinal centers.

Discussion

In the two cases of disseminated necrotizing granulomatosis and angiitis of the lungs with massive splenic necrosis and focal, thrombotic, granulomatous glomerulonephritis described in a previous publication,[1] masses, often subpleural and wedge-shaped, were found in the lungs. A striking feature of these pulmonary masses was the abundant ulceration of the bronchioles and small bronchi which often led to partial or complete obstruction by granulation tissue, neutrophils, and cellular débris. Many of these ulcerated bronchioles and small bronchi became cavities of various sizes. The larger bronchi and trachea were not obstructed, but erosions and ulcerations sometimes were visible in these structures. Numerous vacuolated macrophages filled with cholesterol-rich lipid were present within lumina of persistent alveoli as well as in the alveolar walls and in the adjacent solid granulomatous tissue. An abundant fibroblastic proliferation with collagen deposition was seen forming solid sheets of granulomatous tissue occasionally containing multinucleated giant cells. Variable numbers of eosinophils, plasma cells, lymphocytes, and neutrophils were present in the granulomatous tissue and in the thickened walls of persistent alveoli. Within the granulomatous tissue, some of the arteries and veins were involved in an inflammatory granulomatous or fibrotic process with disintegration of the wall and marked degeneration, reduplication, and defects of the elastic laminae. The intimate association of the vascular changes with the granulomatous tissue was emphasized by the involvement, at the periphery of the granulomatous masses, of segments of blood vessels which directly joined normal segments in the adjacent normal parenchyma. Foci of necrosis surrounded by palisaded cells were fairly abundant in one case but rare in the second. Bacteriologic and fungal studies revealed no causal organisms. Evidence in favor of a hypersensitivity phenomenon as the cause of the lesions in these cases was presented. It is noteworthy that in the published reports of similar cases, many authors, including Klinger,[5] Rössle,[6,7] and Wegener,[8,9] reported extensive ulceration of the respiratory tract.

In one of these cases of disseminated necrotizing granulomatosis, a massively involved lower lobe of the left lung was resected about 2½ months prior to death when no other organs were manifestly involved. The occurrence of this apparently idiopneumonic lesion led to the hypothesis that the hypersensitivity phenomenon responsible for the pulmonary lesions in disseminated necrotizing granulomatosis might be responsible for granulomatous or fibrotic lesions confined solely to the lungs.

It was not difficult to demonstrate this relationship between the separately considered first case of this report, which has already been designated as idiopneumonic necrotizing granulomatosis and angiitis, and the disseminated form. In this idiopneumonic case, many of the important histologic features of the pulmonary lesions of the disseminated form were found, including ulceration and obstruction of the smaller branches of the bronchial tree, macrophages filled with cholesterol-rich lipid, fibroblastic proliferation, focal necrosis with occasional bordering palisaded cells, and marked destruction of the blood vessels. The differences between the idiopneumonic and disseminated forms consisted of the marked collagenization and the obsolete appearance of the vascular changes, both of which appeared to be contemporaneous. It is postulated that an intense reaction of short duration produced a lesion which had become almost completely collagenized, but the involvement of the bronchioles and small bronchi had continued and more recently a reactivation of the process had resulted in focal necrosis within the dense, collagenized fibrous tissue. This separately considered idiopneumonic case may thus be interpreted as an obsolescent lesion of necrotizing granulomatosis with reactivation. The occurrence of asthma in this case is interesting because of the relationship between asthma and necrotizing granulomatosis emphasized by Ehrlich and Romanoff[10] and Churg and Strauss.[11]

A possible relationship between disseminated and idiopneumonic necrotizing granulomatosis and the combined group of pneumonitis of the cholesterol type was suggested by the presence in all three groups of numerous alveolar and intra-alveolar macrophages filled with cholesterol-rich lipid, marked ulceration and obstruction of the smaller branches of the bronchial tree, and subpleural, wedge-shaped masses often well demarcated from the adjacent normal parenchyma. An abundant fibroblastic proliferation and an infiltrate composed of eosinophils, plasma cells, lymphocytes, macrophages, and giant multinucleate cells also were present in the lesions. The idiopneumonic case

of necrotizing granulomatosis was considered a transition type between the disseminated form and the combined groups of pneumonitis of the cholesterol type.

The citation at this point of a case record of the Massachusetts General Hospital[12] is pertinent in that a pulmonary lesion similar to that of my combined group was present, together with a focal, thrombotic, granulomatous glomerulonephritis similar to that found in cases of disseminated necrotizing granulomatosis. It is interesting that Mallory[12] thought that it was not possible to link together the pulmonary and renal lesions but concluded that the pulmonary lesion was an entirely separate chronic pneumonitis of the cholesterol type.

Since it was shown in the previous article[1] that hypersensitivity was most likely responsible for the lesions of disseminated granulomatosis, including the ulceration and obstruction of the smaller branches of the bronchial tree, it was not difficult to visualize a similar but less intense process in pneumonitis of the cholesterol type. The low intensity of the reaction could be due to a smaller quantity of antigen or antibody, or both, and could be responsible for the absence of the destructive angiitis.

It is of interest, however, that certain vascular alterations were found in the combined group of pneumonitis of the cholesterol type. Often, reduplication of the elastic laminae was visible, with thickened, fibrous intimal layers infiltrated by plasma cells and lymphocytes. The lumina were, at times, narrowed. Some of the arteries and veins were completely occluded by organized, recanalized thrombi. Despite the eccentric position of some of the alterations in the elastic laminae and the intimate connection of the vascular alterations in general to the granulomatous tissue so reminiscent of necrotizing granulomatosis, it is recognized that these changes can be found in other inflammatory lesions as secondary phenomena. For example, personal observation of 9 cases of chronic tuberculosis of the lungs, utilizing the elastic stain, revealed similar vascular alterations. Consequently, the vascular alterations in the combined group cannot be accepted entirely as being primarily connected with the fibrotic process.

The agent responsible for the probable sensitization is unknown. Lederer and Rosenblatt[13] found erosions of the trachea in a patient dying of sulfathiazole therapy and the wide use of the sulfonamides would make them suspect. The histories of the patients of the combined group, however, yielded little information regarding the possible rôle played by the sulfonamides. Only two of my patients belonging

to the combined group had taken sulfonamides, one prior to the onset of symptoms and one following the onset of symptoms. Robbins and Sniffen[2] could not incriminate the sulfonamides in their cases, and Chiari[4] did not mention them.

The lack of specific bacteriologic or fungal pathogenic agents in necrotizing granulomatosis, which supports the hypothesis of a hypersensitivity phenomenon, was duplicated in my combined group of pneumonitis of the cholesterol type, and Robbins and Sniffen[2] found in their cases of pneumonitis of the cholesterol type that bacteriologic staining of tissues was uninformative. Chiari,[4] likewise, obtained inconclusive results from bacteriologic studies. The nondescript bacteria that have been found must be considered secondary invaders of the necrotic débris obstructing the smaller branches of the bronchial tree.

In a consideration of the mechanism by which the parenchymal changes are produced in pneumonitis of the cholesterol type, it is of interest that Robbins and Sniffen[2] pointed out quite clearly that the histologic changes in their cases of chronic pneumonitis of the cholesterol type were identical with those found following obstruction of a major bronchus. Personal observations of one case in which obstruction of a major bronchus was due to a mediastinal cyst and of 2 cases in which the obstruction was due to carcinoma of the bronchus confirmed the similarity of the changes. It is important, however, to note that Robbins and Sniffen found no obstruction of the major bronchi in their cases of pneumonitis of the cholesterol type and this is corroborated with regard to the cases of this report on the basis of bronchoscopic examinations and of direct observation of specimens. Furthermore, Robbins and Sniffen conceded that the ulceration and obstruction of the smaller branches of the bronchial tree may have been a factor in their cases, but they could not explain satisfactorily the pathogenesis of the lesions in these small branches and came to the conclusion that the lesions in the pulmonary parenchyma were the result of an attempt by the lung to dispose of precipitated cholesterol ester, assuming an endogenous origin of cholesterol in the lung due to some unknown factor or factors. No evidence of lymphatic obstruction was seen in their cases. Chiari,[4] too, found similar alterations in the smaller branches of the bronchial tree but believed that the foam cells were produced by obstruction of lymphatic channels.

Gross, Brown, and Hatch[14] recently administered antimony trioxide by inhalation to rats and produced a lipid pneumonia. No ulcerations of the bronchioles or bronchi were observed. These authors suggested

that certain clinical lipid pneumonias might be related to endogenous lipids on the basis of these experiments. Ross[15] previously had observed the appearance of intra-alveolar macrophages following the introduction of toxic and non-toxic substances into the lungs of rabbits.

It is my contention, however, on the basis of the present study, that the primary disorder in pneumonitis of the cholesterol type is the ulcerative obstructive lesions of the smaller branches of the bronchial tree, which produce changes in the pulmonary parenchyma similar to those found following obstruction of a major bronchus, and not the endogenous production and elimination of cholesterol within the pulmonary parenchyma as Robbins and Sniffen believed. No obstruction of lymphatic channels could be found in my cases of pneumonitis of the cholesterol type.

As for the work of Gross, Brown, and Hatch,[14] it is difficult to see any relationship between their experiments and the vacuolated macrophages of disseminated and idiopneumonic necrotizing granulomatosis and of pneumonitis of the cholesterol type, in which there is no evidence that a toxic substance like antimony trioxide is present. In obstruction of the smaller branches of the bronchial tree, as in cases with obstruction of a major bronchus, the simplest explanation is that a lack of oxygen is responsible for producing the lipid-filled macrophages whether they come from the lining cells or the alveolar walls. Whether a toxic substance or lack of oxygen produces these cells, once they are present within the alveoli a deranged metabolism, due to a strange and unsuitable environment, could easily lead to the production of visible intracytoplasmic lipid.

The cause of the ulcerative lesions is considered to be a hypersensitivity phenomenon and one might refer to these lesions in pneumonitis of the cholesterol type as an Arthus reaction localized in the bronchial tree. That some fibrosis may be produced by the cholesterol-rich lipid liberated from the disintegrating macrophages cannot be denied, but this also must be considered a secondary phenomenon. The possibility that there may be a direct primary effect on the fibroblasts leading to a massive production of fibrous tissue similar to the occurrence in the pulmonary lesions of necrotizing granulomatosis must be kept in mind.

Summary

Six cases of chronic pneumonitis of the cholesterol type and one case of idiopneumonic necrotizing granulomatosis and angiitis have been compared with 2 cases of disseminated necrotizing granulomatosis and

angiitis. In all of these cases, the lesions of the lungs, often in the form of subpleural masses, included ulceration and obstruction of the bronchioles and small bronchi, fibroblastic proliferation, collagen deposition, and vacuolated macrophages filled with cholesterol-rich lipid. The case of idiopneumonic necrotizing granulomatosis appeared to be a transition between the cases of chronic pneumonitis of the cholesterol type and the cases of disseminated necrotizing granulomatosis and angiitis.

The hypothesis is offered that the primary disorder in pneumonitis of the cholesterol type is the ulceration and obstruction of the smaller branches of the bronchial tree, which bring about the appearance of the intra-alveolar macrophages filled with cholesterol-rich lipid, the latter being a secondary phenomenon. Furthermore, it is suggested that these changes in the smaller branches of the bronchial tree are caused by a hypersensitivity phenomenon, similar, but of lower intensity, to that responsible for like changes in both idiopneumonic and disseminated necrotizing granulomatosis and angiitis, and that this represents an Arthus reaction localized in the bronchial tree. The cause of the sensitization is unknown.

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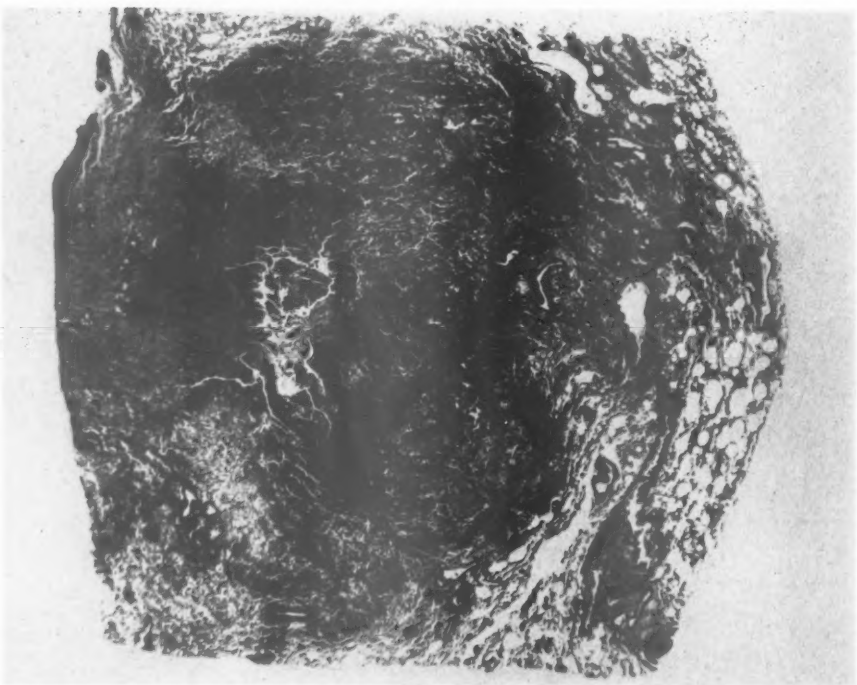
[*Illustrations follow*]

LEGENDS FOR FIGURES

FIG. 1. Case 1. Lung. Disseminated necrotizing granulomatosis and angiitis. Subpleural, wedge-shaped mass of granulomatous tissue and collagen with central cavitation found at necropsy. The pleural surface is on the left. Masson's trichrome stain. × 7.5.

FIG. 2. Case 1. Disseminated necrotizing granulomatosis and angiitis. Surgically resected lower half of left lung. Marked ulceration of bronchiole with partial replacement of wall by granulomatous tissue containing multinucleated giant cells. Similar granulomatous tissue has obstructed the lumen. Hematoxylin and eosin stain. × 125.

FIG. 3. Case 1. Lung. Disseminated necrotizing granulomatosis and angiitis. Surgically resected lower half of left lung. Numerous vacuolated macrophages filled with Sudan IV positive, cholesterol-rich lipid in lumina of alveoli. For comparison with Figure 6. Hematoxylin and eosin stain. × 125.



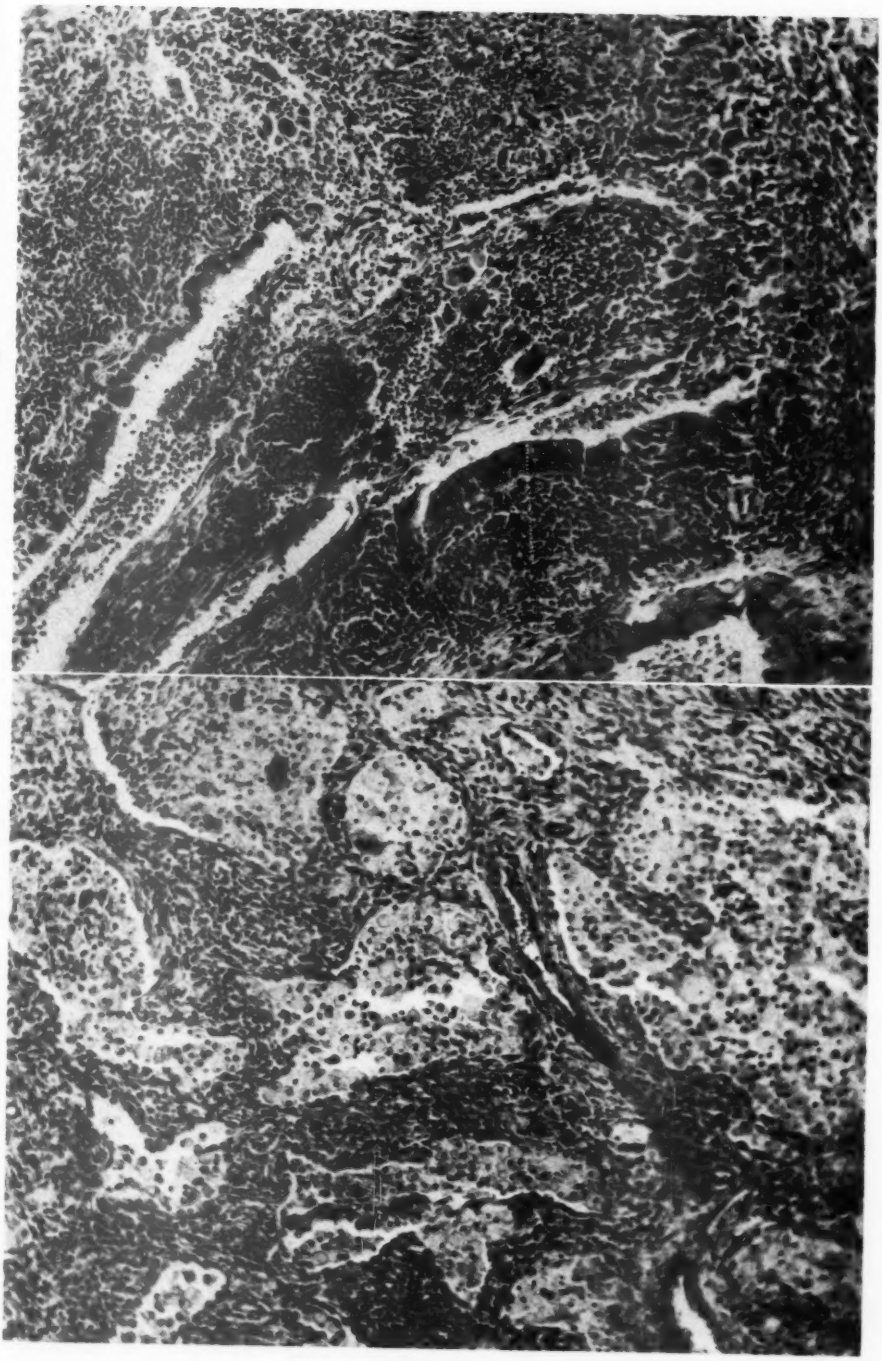


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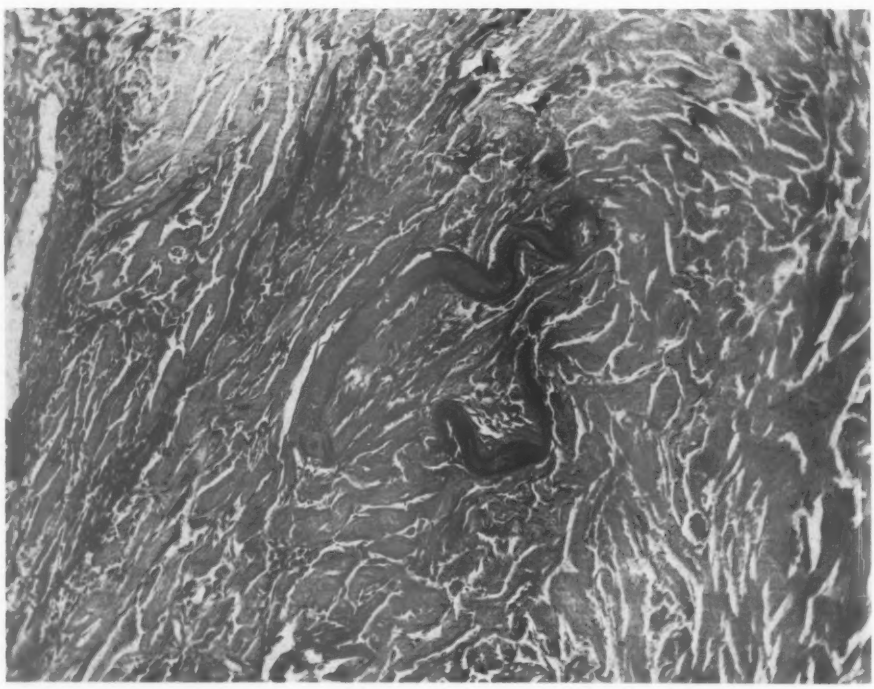
FIG. 4. Idiopneumonic obsolescent necrotizing granulomatosis and angiitis. Artery can be recognized only with elastic tissue stain by persistent remnants of elastic tissue laminae. Lumen filled with acellular, collagenized fibrous tissue similar to that found surrounding the artery. Verhoeff's elastic tissue stain. × 125.

FIG. 5. Combined group. Pneumonitis of the cholesterol type. Marked ulceration of small bronchus with only remnant of mucosa present. Obstruction of lumen by cellular débris may be noted. Hematoxylin and eosin stain. × 125.

FIG. 6. Combined group. Pneumonitis of the cholesterol type. Numerous vacuolated macrophages filled with Sudan IV-positive, cholesterol-rich lipid in lumina of alveoli and in thickened alveolar walls. For comparison with Figure 3. Hematoxylin and eosin stain. × 125.



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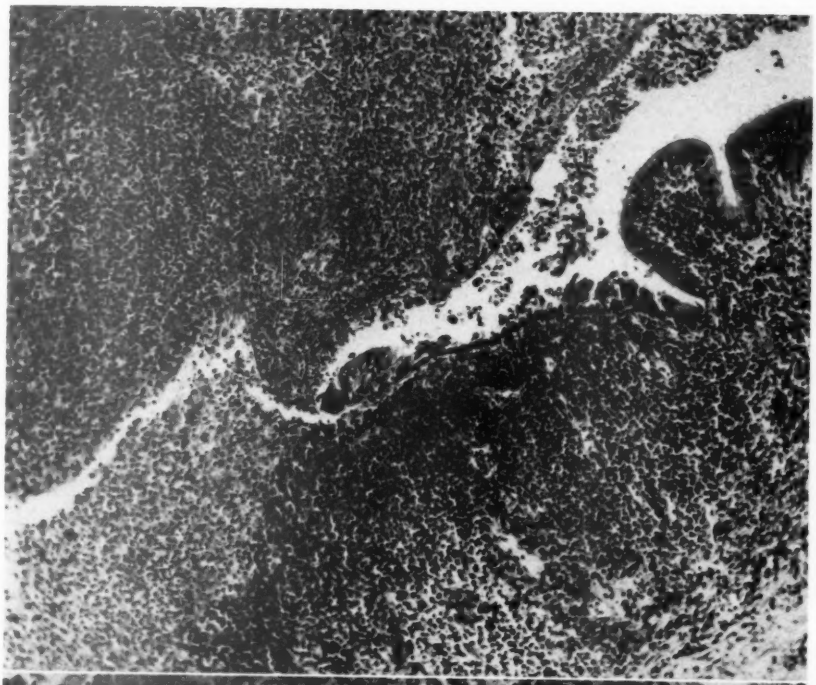

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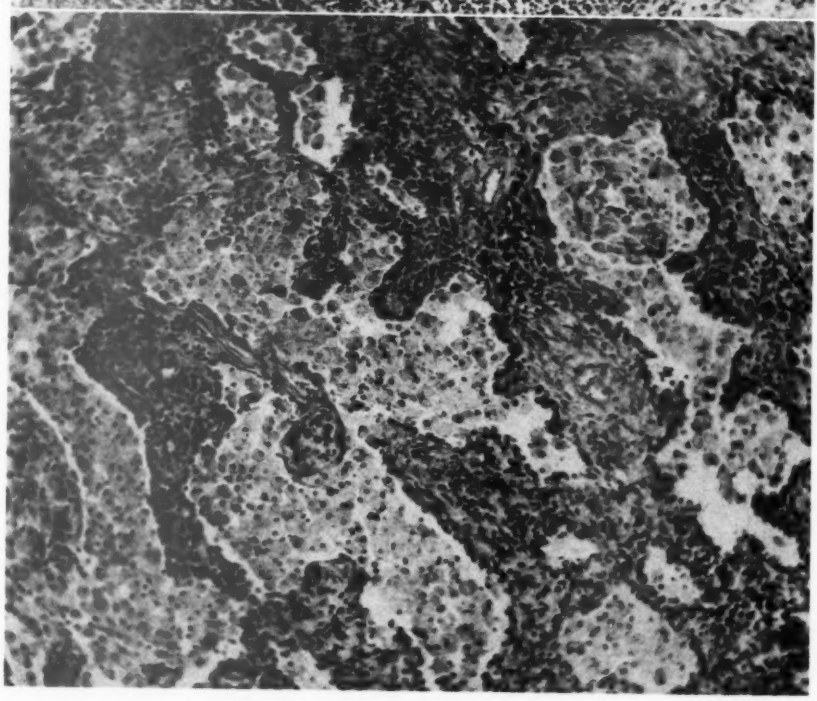

HISTOPATHOLOGY OF HOST-INDUCED ALTERATIONS IN THE STRAIN SPECIFICITY OF SARCOMA I IN MICE *

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Previous studies by Casey[1,2] and Kaliss and Snell[3] have demonstrated that prior intra-abdominal injection of frozen-dried (lyophilized) cancerous and normal tissues in both rabbits and mice would enhance the growth of an implanted tumor which would not ordinarily grow in the host. The lyophilized tissue injected by these investigators was tissue taken from the usual host animal in which the tumor implant would grow without any prior treatment.

Subsequent studies by our own group[4] have demonstrated that, if mice are under cortisone treatment and are concurrently treated with lyophilized neoplastic tissue, not only will a live implant grow in an ordinarily non-susceptible host, but the enhancement will be so increased that intra-abdominal and occasionally intrathoracic metastases appear. In all instances the live tumor implant was the same tumor which was injected as a lyophilized preparation in the pre-treatment of the mice.

In order to understand how this phenomenon affects the host and the live tumor, further studies are being conducted. This report concerns itself with the growth characteristics of the intraperitoneal tumor metastases which arise under the conditions described.

EXPERIMENTAL PROCEDURE AND FINDINGS

The nomenclature for designating inbred mouse strains herein mentioned is taken from the *Standardized Nomenclature for Inbred Strains of Mice.*[5]†

The tumor used is mouse tumor, Sarcoma I. It is a sarcoma which originated at the University of California in 1947. On December 3, 1951, the tumor was in its 152nd transplant generation at the Jackson

* Portions of this work were performed at the Department of Laboratories and Research of Westchester County, Valhalla, N.Y., and the Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Me.

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† All mice were obtained from the Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Me.

laboratories from which it was obtained, and is now carried in "A" strain mice in our laboratories. This tumor grows in 100 per cent of "A" strain mice and hybrids of "A" strain, both male and female, and is palpable in 2 to 3 days; animals so implanted die with a large tumor mass within 3 weeks. Heretofore the tumor has not been observed nor reported to metastasize. The sarcoma is composed of solid, dense sheets of spindle-shaped and polyhedral tumor cells, mitotic figures are numerous, and the stroma is extremely scant. The polyhedral cells predominate.

C57BL/6 mice* were treated previously with cortisone and continued on cortisone throughout the experiment at a level which would produce grossly discernible debility (0.1 to 1.0 mg. daily). Three injections of 15 mg. each of lyophilized Sarcoma I, which was re-suspended and homogenized in saline solution prior to each intraperitoneal injection, were administered at 5-day intervals. Ten days after the last injection of lyophilized tissue suspension, a fragment of fresh Sarcoma I from growth in an untreated "A" strain mouse was implanted subcutaneously in the suprascapular region. On the 16th and 20th days post-implantation, these animals were sacrificed and fragments were taken from the intraperitoneal sarcoma that had developed in these animals. These fragments, by means of a trocar, were then implanted subcutaneously in the suprascapular region in six groups of untreated normal mice of differing genetic strains, with five mice in each group. These six groups represented one strain in which the tumor ordinarily grows (indigenous) and 5 (non-indigenous) strains in which this tumor ordinarily does not grow, or in which, if growth appears, the tumor regresses within 5 days of implantation. The indigenous strain was A/Jax, an "A" strain mouse. The non-indigenous strains were C3H, C57BL/6, DBA/1, C57BR/cd, and ST (mouse strains in which Sarcoma I does not grow on implantation in untreated animals).

The subcutaneous implants of the metastatic Sarcoma I from the peritoneum of the treated C57BL/6 mice grew progressively and when sizes of 1.5 to 3.0 cm. were reached by the tenth day after implantation, these animals were sacrificed and fragments of these tumors were then re-implanted into each respective strain of untreated normal mice (five per group), *e.g.*, the growths on DBA/1 mice were transplanted to fresh DBA/1 mice, etc. The remainder of the tumors were saved for histologic examination. The second and subsequent third generation implants were obtained from mice of each strain, respectively,

* This strain is not an "A" strain nor a hybrid "A" strain.

and sacrificed 16 days after implantation. In all instances, progressively enlarging, solid tumors were present. In some, there were central areas of necrosis, which is characteristic for this tumor, even when it grows in its indigenous "A" strain of mice. Since this study was primarily concerned with the histopathology of these tumors, the animals were not carried for a sufficiently long period of time to study the question of survival or whether or not these tumors eventually would have regressed. These aspects are currently under investigation.

In addition to the foregoing procedure, 0.5 ml. of ascitic fluid from one of the original C57BL/6 mice which was treated with cortisone, lyophilized sarcoma, and a live implant, and in which intraperitoneal metastases had developed, was injected intraperitoneally into each of 6 untreated and normal mice of the same six strains. These animals were sacrificed 23 days after the injection of the ascitic fluid. The abdomens were markedly distended. The mice were in a debilitated condition and had difficulty in moving about (Fig. 1). Examination revealed extensive intraperitoneal sarcomatosis, which was confirmed by microscopic examination.

Microscopic examination of the original Sarcoma I from "A" strain mice, the original intraperitoneal metastases in the treated C57BL/6 mice, and also the tumor in the subsequently untreated animals revealed significant differences (Figs. 2 to 8). Both the original sarcoma (from "A" strain) and the intraperitoneal metastases in the treated C57BL/6 animals revealed a fairly uniform histologic picture. The cells were for the most part oval to polygonal with occasional spindle forms. The nuclei were uniformly vesicular and revealed relatively similar chromatin patterns. Giant cells were scarce. The size of the cells was rather uniform. The cells were closely packed and very little stroma was present. In contrast, the tumors that grew in the untreated normal indigenous and non-indigenous strains revealed marked differences in the size and shape of the cells with many atypical forms present. Spindle cells were numerous. Giant tumor cells were seen more frequently. The nuclei varied considerably. Many cells exhibited extreme degrees of hyperchromatism. The cells were more loosely packed and stroma appeared to be somewhat more abundant. Mitotic figures were more numerous. The difference in structure indicated a much greater degree of anaplasia in the latter tumors than in the former.

It is apparent that the alteration of the original host responses, influenced by prior treatment with cortisone and lyophilized sarcoma, resulted in relative alterations in the growth characteristics and strain specificity of the original Sarcoma I. This was manifested by a changed

histologic appearance in the direction of anaplasia. This study is currently being repeated in the indigenous A-strain mice to determine whether host-induced alterations will similarly affect Sarcoma I when it grows in its indigenous host. Whether or not this relative change in both growth characteristics and strain specificity is due to natural selection of the more virulent cells in the tumor or is a change that is actually induced in the tumor itself remains to be determined.

Summary

Sarcoma I grafts from intra-abdominal metastases in C57BL/6 mice treated with cortisone, lyophilized Sarcoma I, and a live implant, transplanted to five different strains of mice in which ordinarily this tumor will not grow, have grown progressively thus far in three successive generations.

The histopathology of these tumors revealed marked anaplasia as compared to the original tumor.

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FIG. 1. Gross photograph of an A/Cloudman mouse (right) and C57BL/6 Jax mouse (left) with intraperitoneal sarcoma, 23 days after inoculation with ascitic fluid (Sarcoma I) containing tumor cells obtained from previously treated mice.

FIG. 2. Photomicrograph of original Sarcoma I growing subcutaneously in an A/Cloudman mouse. Hematoxylin and eosin stain. × 720.

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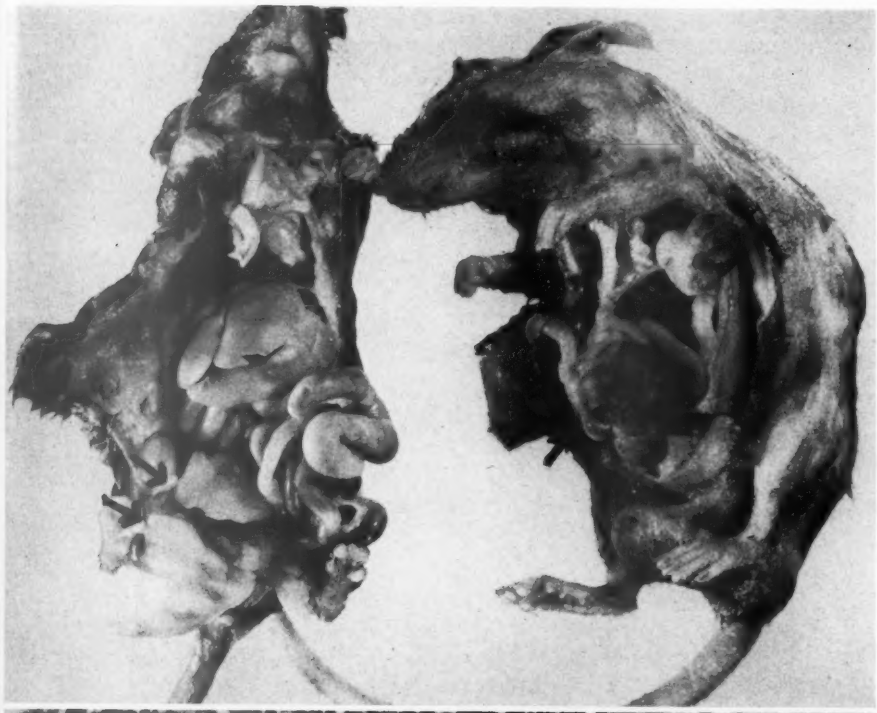


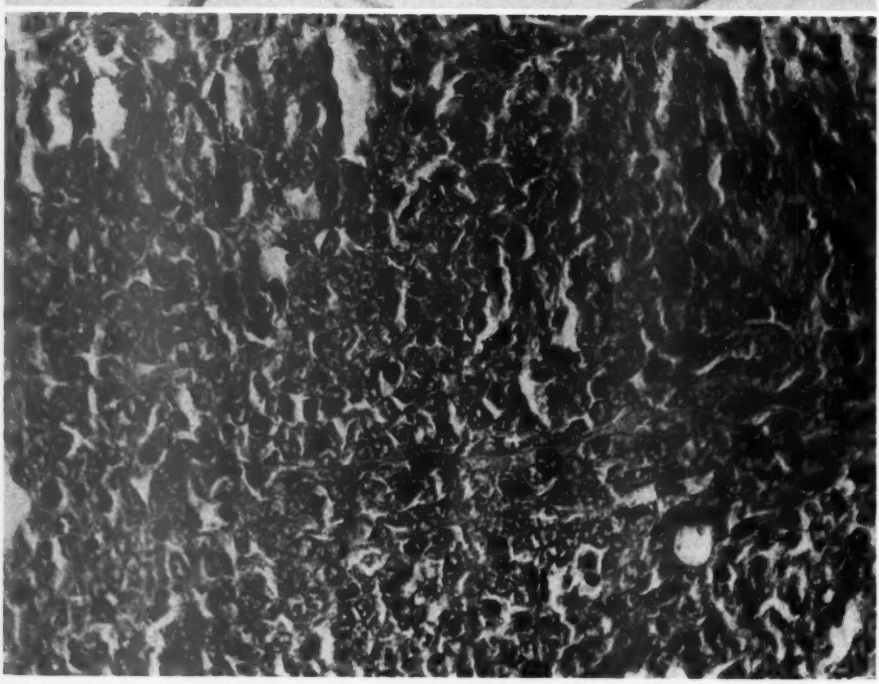
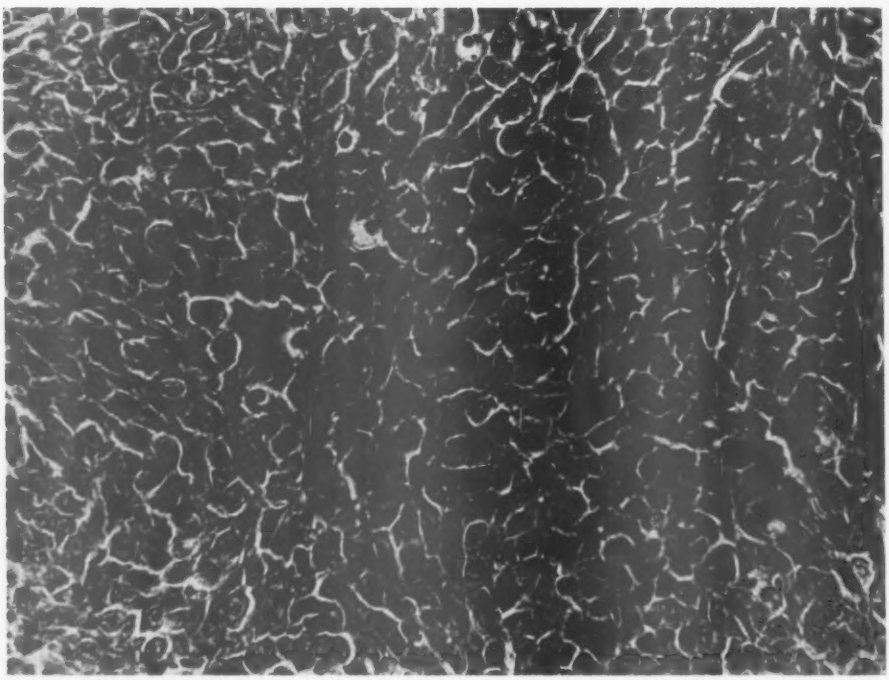
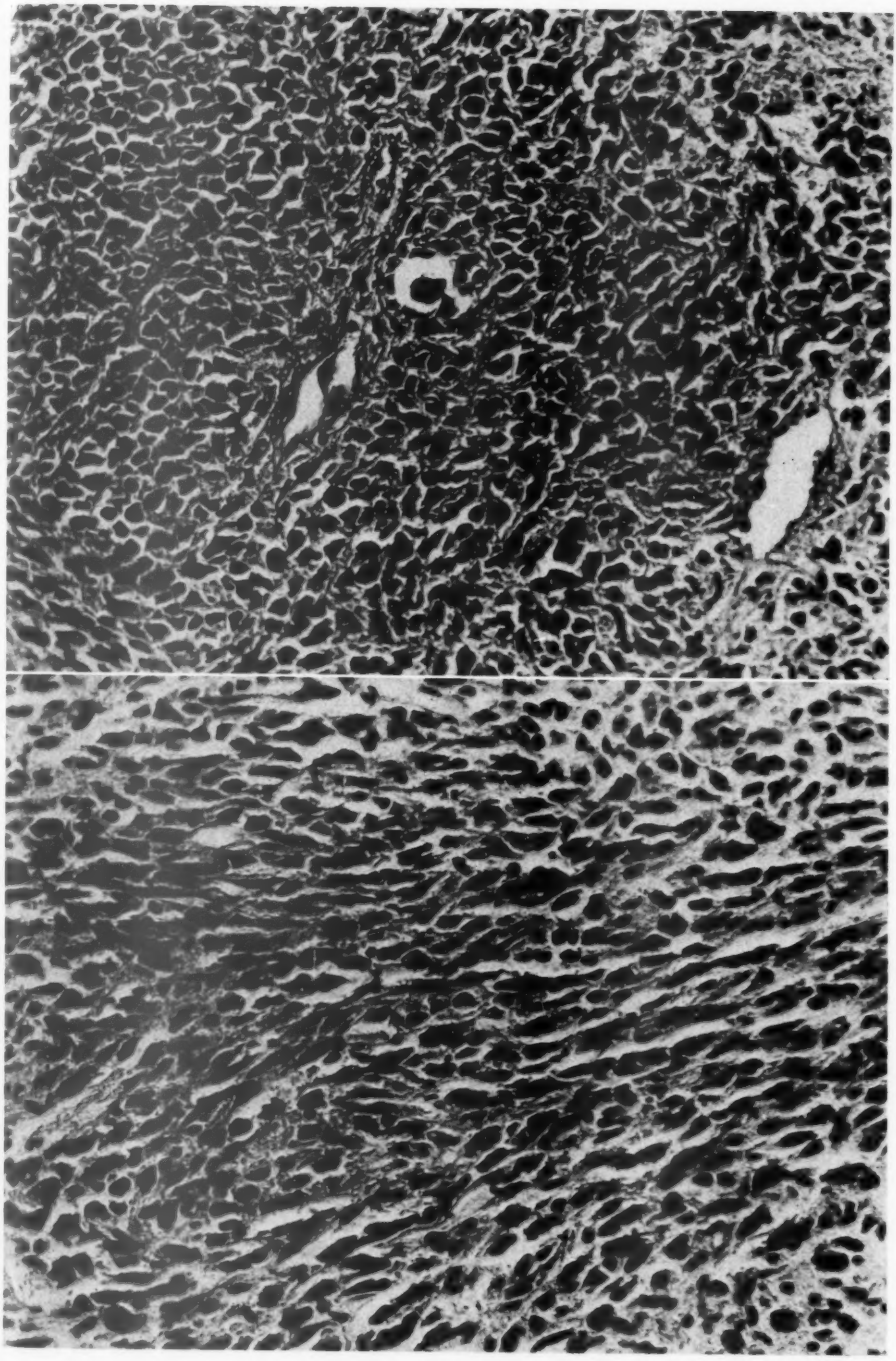


FIG. 3. Photomicrograph of intraperitoneal sarcoma in previously treated C57BL/6 Jax mouse. Hematoxylin and eosin stain. × 720.

FIGS. 4 and 5. Photomicrographs of tumor growing in two untreated non-indigenous strains of mice. Hematoxylin and eosin stain. × 720.





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FIGS. 6 to 8. Photomicrographs of tumor growing in three additional untreated non-indigenous strains of mice. Hematoxylin and eosin stain. × 720.

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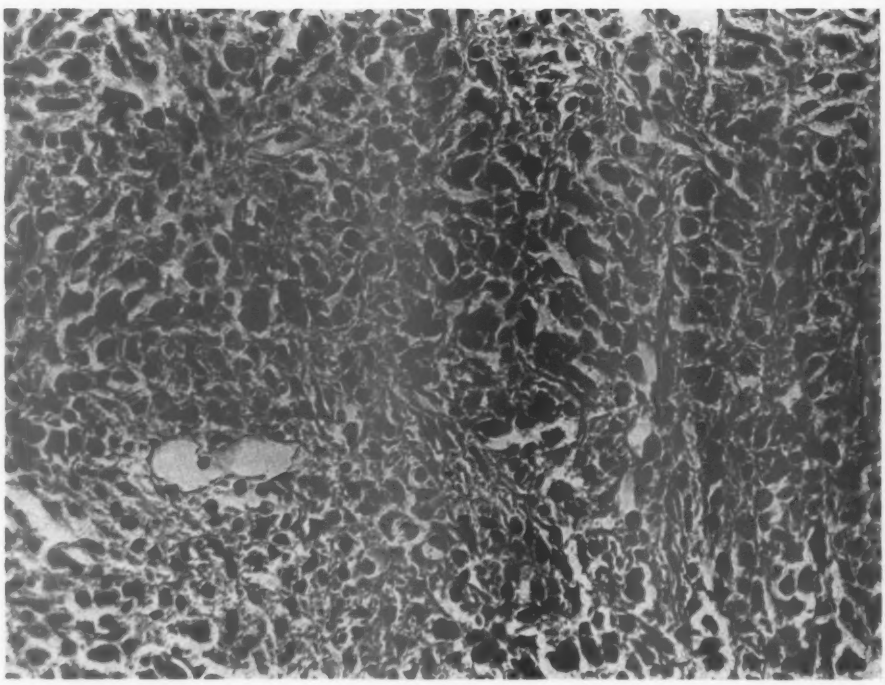

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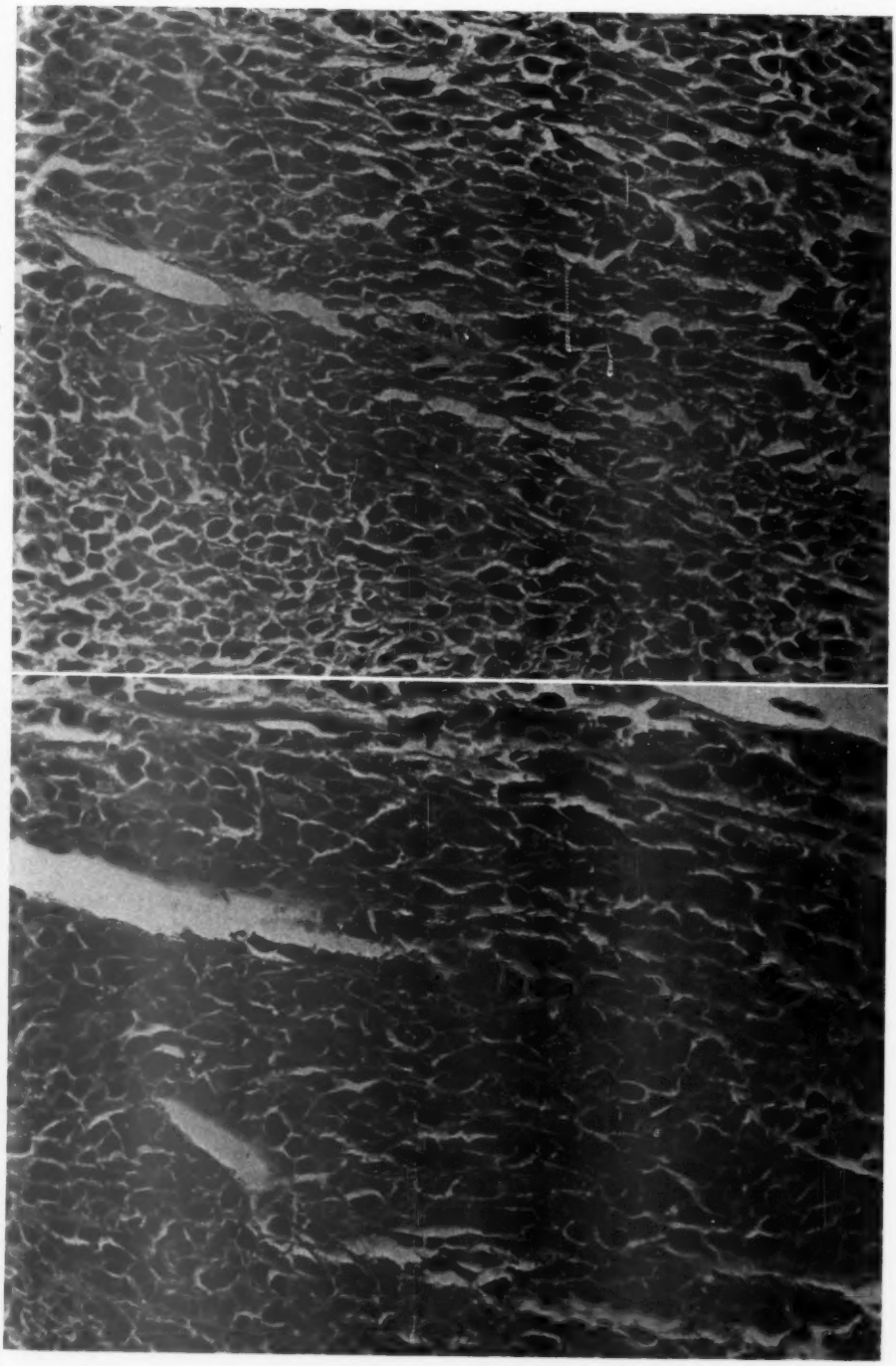

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THE EFFECTS OF ACTH AND CORTISONE UPON SUSCEPTIBILITY TO TRICHINOSIS IN MICE*

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Recently Davis and Most[1] and Luougo, Reid, and Weiss[2] reported upon the use of ACTH in human trichinella spiralis infections and Rosen[3] upon the use of cortisone in one case of human trichinosis. Their patients improved clinically following the administration of these agents and the authors recommended further investigation of these drugs in similarly infected patients.

Luougo *et al.*[2] included with their 3 case reports the results of experimental infections in guinea-pigs. They found no alteration in host response or decrease in mortality following ACTH.

In view of these reports and the desirability of further experimental work in animals to elucidate this problem, a series of experiments on the effects of ACTH and cortisone upon susceptibility to experimental trichinosis in mice was initiated. The degree of susceptibility was measured by the morbidity and mortality associated with the experimental infections. No attempt was made to determine the number of larvae produced in treated and untreated mice. Additional experiments concerning the effects of ACTH and cortisone on immunity were carried out and will be reported upon later.

MATERIALS AND METHODS

Swiss mice from our own colony, which is free of salmonella and metazoan parasites, were employed as experimental hosts. They were 6 to 8 weeks of age at the time of infection. The trichinella larvae used for infective doses were isolated by pepsin digestion from stock mice with infections of 6 weeks' duration. Infective doses were determined by the dilution count method described by McCoy[4] and concentrated in conical centrifuge tubes. With the aid of a rubber bulb, the larvae in about 1 cc. of tap water were drawn into a tapered, small bore pipette. While the mice were under ether anesthesia, the infective dose was administered directly into the stomach by passage of the pipette down the esophagus. All animals were given infective doses on the basis of larvae per gram (lpg) of body weight.

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ACTH (Armour) was diluted in 0.85 per cent saline solution, pH 7.1, and administered in 0.1 ml. injections subcutaneously. Cortisone acetate (Merck) was diluted with aqueous vehicle control and given in 0.1 ml. injections subcutaneously.

The specific procedures employed are discussed with their respective experiments which are designated as follows:

1. Effects of ACTH upon susceptibility
2. Effects of combined penicillin and dihydrostreptomycin with ACTH upon susceptibility
3. Effects of cortisone upon susceptibility
4. Effects of different doses of cortisone alone and with combiotic* upon susceptibility

Experiment 1. Effects of ACTH upon Susceptibility

Several experiments were carried out to determine the effect of daily administration of 0.25 mg. of ACTH upon 50 and 75 lpg infections in mice. ACTH treatment was started 1 day post-infection and continued for 30 days. The results from five experiments are summarized in Table I. The data indicate that the infective doses constituted an LD_{80} within a 30-day period. Daily subcutaneous injection of 0.25

TABLE I
Effects of ACTH upon Mortality

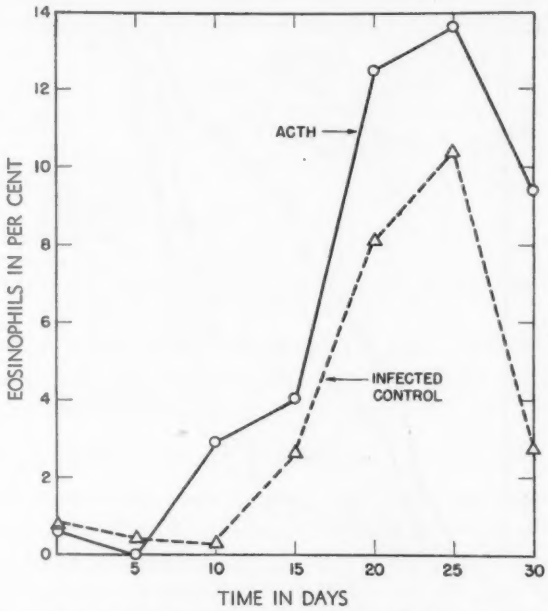
Group description	No. of mice	No. which died	Per cent which died
1. Normal controls	90	0	0
2. ACTH controls	62	0	0
3. Trichinella infected controls	160	132	82.8
4. Trichinella infected and treated with ACTH	162	150	92.5

mg. of ACTH produced a slight increase in susceptibility to trichinella infections in mice.

On the basis of mg. per gm. of body weight, the daily dose of 0.25 mg. of ACTH used in mice was three times the level used by Davis and Most[1] and sixteen times the dose employed by Luougo *et al.*[2] in man. Our ACTH dose was four times less than that employed by Luougo *et al.* in guinea-pigs. Lowering of the number of circulating eosinophils has been used as a test of the capacity of the adrenal cortex to respond to ACTH stimulation. In order to determine the effects of

* Charles Pfizer & Co., Brooklyn, N.Y.; combination of penicillin and dihydrostreptomycin.

ACTH upon the characteristic eosinophilia associated with a trichinella infection, differential white cell counts were determined at 5-day intervals throughout the 30-day period of observation. It may be noted from Text-figure 1 that daily dosage of 0.25 mg. of ACTH did not alter the characteristic eosinophilia found with a trichinella infection in mice. The temporary depression of circulating eosinophils appears indicative of adequate adrenal-cortical response. Luougo *et al.* reported an initial drop in circulating eosinophils in infected guinea-



Text-fig. 1. Effects of ACTH upon circulating eosinophils in trichinella-infected Swiss mice.

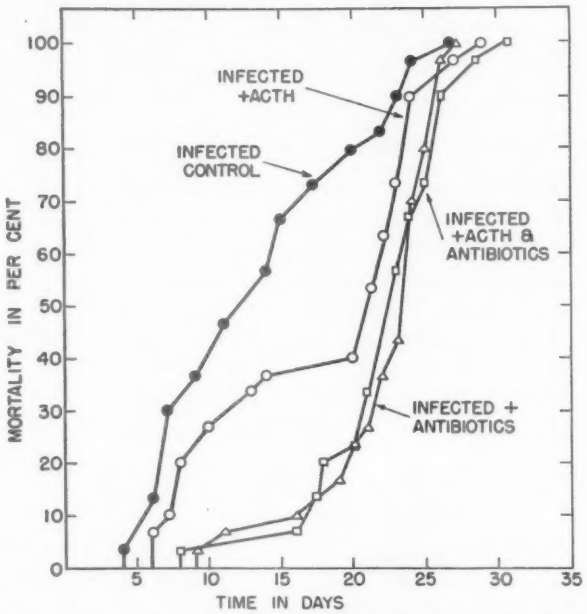
pigs followed by a gradual rise after the 3rd day, reaching a maximum on the 24th day of infection. Precipitin tests of serum from infected control mice and ACTH-infected mice on the 25th day of infection exhibited an equal level in the ability to precipitate dilutions of precipitinogen prepared from trichinella larvae.

Pathologic Findings. Two mice were killed at 5-day intervals during the course of the infection. Sections of all organs were made and stained with hematoxylin and eosin. Study of these sections revealed no changes which would enable one to differentiate the ACTH-treated from the untreated group, either by degree or type of inflammatory

infiltrate or the number of parasites. Luougo *et al.*[2] were able to demonstrate no apparent difference between the lesions from treated and untreated animals. The numbers of encysted larvae in treated and untreated animals receiving equivalent infective doses were similar.

Experiment 2. Effects of Antibiotics and ACTH upon Susceptibility

A number of papers have appeared concerning the adverse effects of ACTH and cortisone therapy in certain bacterial infections. An



Text-fig. 2. Effect of ACTH and antibiotics upon mortality in trichinella infections in mice.

excellent discussion of this literature may be found in the review by Thomas.[5] In view of the concomitant secondary bacterial invasion during the intestinal phase of a trichinella infection,[6] an experiment was carried out to see if antibiotic therapy combined with ACTH altered the mortality in an LD_{100} trichinella infection in mice. One of us[6] has previously shown that the mortality observed during the intestinal phase of a severe infection was delayed several weeks when secondary infections were controlled with antibiotics. However, this later resulted in an increase in the number of deaths during the muscle invasion by the newly liberated trichinella larvae in the third, fourth, and fifth weeks of the infection.

This experiment was designed to test the effects of daily subcutaneous administration of 1,500 units of crystalline penicillin-G (Wycillin), 1,250 μg. of dihydrostreptomycin sulfate, and 0.25 mg. of ACTH upon the mortality produced by a 75 lpg trichinella infection in 8-weeks-old mice. The data are plotted in percentage of mortality against time in Text-figure 2. A delay in the number of deaths may be noted during the intestinal phase of the infection in the antibiotic infected control group and in the antibiotic and ACTH experimental group. During the period of muscle invasion, a sharp increase in the mortality may be seen in these two groups. It is evident that the antibiotic control of the bacteremia during the initial phase of the trichinella infection did not alter the final mortality observed with a severe infection of trichinosis in mice.

TABLE II
Effects of Cortisone upon Susceptibility

Group description	No. of mice	No. which died	Per cent which died
1. Cortisone controls	47	0	0
2. Trichinella infected* controls	99	51	51.5
3. Trichinella infected, with cortisone	99	98	99.0

* Infective doses constituted 50 to 65 larvae per gm.

Experiment 3. Effects of Cortisone upon Susceptibility

Several experiments were carried out to determine the effects of cortisone acetate (Merck) upon experimental infections of *Trichinella spiralis* in mice. The treated groups were given daily 0.5 mg. of cortisone acetate subcutaneously for 30 days. All dilutions of cortisone were made up with aqueous vehicle no. 1.* The cortisone therapy was started 1 day post-infection. Since a slight increase in susceptibility to trichinella infection was observed with ACTH therapy, infective doses of 50 to 65 lpg were used in these experiments. As shown in Table II, the infective doses constituted an LD_{50} infection in mice within a 30-day period of observation. It is evident from these data that daily administration of 0.5 mg. of cortisone resulted in an increase in susceptibility of over 45 per cent mortality with an LD_{50} infective dose of *T. spiralis* in mice.

Differential white cell counts were recorded at 5-day intervals during the course of the infection. A significant depression of circulating

* We are indebted to Dr. Elmer Alpert, Medical Division, Merck & Co., Inc., Rahway, N.J., for a supply of aqueous vehicle no. 1.

eosinophils was observed in both non-infected cortisone controls and in trichinella-infected mice given the 0.5 mg. dose of cortisone. The inhibition of a characteristic eosinophilia may or may not be due to the cortisone, inasmuch as it has been observed[6] that severe infections of trichinella in mice may not be accompanied by eosinophilia. Howard,[7] in 1899, first reported the absence of eosinophilia in a fatal case of trichinosis in man. In the present experiment, lymphopenia accompanied by neutrophilia was noted in the third week of infection in the cortisone-treated group. At this time the majority of the mice were moribund. Thus the absence of a rise in eosinophils may be due to the severity of the infection instead of the cortisone therapy.

Experiment 4. Effects of Different Doses of Cortisone and Combiotic upon Susceptibility

In view of the significant increase in susceptibility to trichinella infection of mice treated with 0.5 mg. of cortisone daily, an experiment was designed to test the effects of daily administration of 0.1 and 1.0 mg. of cortisone upon susceptibility. As in the previous experiments, the cortisone was given subcutaneously for 30 days. The data are shown in Table III. The infective dose of larvae in this ex-

TABLE III
Effects of Different Doses of Cortisone on Mortality

Group description	No. of mice	No. which died	Per cent which died
1. Cortisone control, 1.0 mg. daily	20	1	5
2. Infected control	30	30	100
3. Infected*+1.0 mg. of cortisone	30	30	100
4. Infected+0.1 mg. of cortisone	30	30	100
5. Infected+aqueous vehicle no. 1	20	20	100

* Infective doses were 65 larvae per gm.

periment constituted an LD_{100}. The infected mice in group 3 treated with 1.0 mg. of cortisone died within the 12th and 21st day of infection. A majority of the infected control animals died within the 18th to 30th day of infection. Groups 4 (0.1 mg. of cortisone) and 5 (aqueous vehicle control) died during the same interval as described for the infected untreated control group 2.

An antibiotic control experiment was undertaken using the 0.1 and 1.0 mg. cortisone levels with a severe infection of trichinella. Combiotic (Pfizer) containing 2,000 units of crystalline penicillin and

2,500 μg. of dihydrostreptomycin sulfate was given daily with the stated levels of cortisone. The results are shown in Table IV. There was no decrease in mortality in the 30-day period of observation. An initial delay in the number of deaths again was found with infected controls given combiotic. A similar lag in the number of deaths during the intestinal phase was observed in the group given combiotic and 0.1 mg. of cortisone. This was not found when 1.0 mg. of cortisone was given with the combiotic. Thus, in group 3 all animals died before the majority of the infected controls died.

Summary and Conclusions

From these observations, it is evident that ACTH and cortisone do not favorably alter the course of experimental trichinosis in mice. A slight increase in susceptibility to trichinella infections was noted following treatment with ACTH.

Table IV

Effects of Different Doses of Cortisone and Combiotic on Mortality

Group description	No. of mice	No. which died	Per cent which died
1. Infected control	30	30	100
2. Infected+combiotic	20	20	100
3. Infected*+1.0 mg. of cortisone and combiotic	30	30	100
4. Infected+0.1 mg. of cortisone and combiotic	30	30	100

* Infective doses were 65 larvae per gm.

A characteristic eosinophilia appeared during the third week of infection in both the infected control group and the ACTH-treated group. An equal level of precipitins for trichinella precipitinogen was found for ACTH-treated mice and infected controls on the 25th day of the infection. Pathologic study revealed no significant changes either in degree or type of inflammatory infiltration in treated and untreated animals or in the number of encysted parasites. Antibiotic control of concomitant bacterial infection combined with ACTH did not decrease the final mortality observed with the experimental trichinella infections.

Cortisone therapy in trichinella-infected mice produced a significant increase in susceptibility as shown by a 45 per cent increase in mortality. Eosinophilia did not appear in cortisone-treated infected animals. Antibiotic control of secondary bacterial infections with

combiotic, combined with various doses of cortisone, did not decrease the final mortality in trichinella-infected mice.

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KERATOMALACIA AND PANOPHTHALMITIS IN "YELLOW" MICE *

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In "yellow" strains of mice, individuals with yellow coat color show hereditary polyphagia and, if fed proper diets, develop obesity and diabetes, while their littermates with gray coat color are free of these traits. In the obese individuals, hyperglycemia is associated with insulin resistance and decrease in hepatic glycogen; the pancreatic islets are hyperplastic and hypertrophic but, so far, no microscopic changes or functional disturbances could be demonstrated in adrenal and thyroid glands, or testicles.[1-6]

In our colony of yellow mice raised for the purpose of skeletal studies, characteristic ocular lesions were encountered,[7] the features of which will be described.

MATERIAL AND METHODS

Our colony of yellow mice—strain YBR/Wi—was raised from a litter of 5 obtained from Dr. J. Walter Wilson of Brown University. The breeding animals were kept on a stock diet of Purina laboratory chow and water *ad libitum*. After weaning, individuals of both sexes and of gray as well as of yellow coat color were fed different diets. One group of animals received the regular stock diet of laboratory chow; another group was fed a fat-enriched diet containing 25 per cent lard (Swift's silverleaf brand), and another group was kept on a carbohydrate-enriched diet containing 56 per cent cornstarch added to the stock diet. The fat-enriched diet thus contained about 30 per cent fat and the high-carbohydrate diet about 79 per cent carbohydrates. All diets were adequate in minerals and vitamins according to the minimum requirements established for mice of other strains.[8,9]

The present report deals with observations made on 252 mice: 62 gray males, 49 yellow males, 63 gray females, and 78 yellow females. Details can be seen from Tables I and II. The diets were supplied in *ad libitum* amounts, and no attempt was made to determine the food intake, since such studies have been carried out previously.[2,3,10] For

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microscopic study, the eyes, including parts of the lids, were removed as a whole, fixed in Bouin's solution, dehydrated slowly, and embedded in a mixture of celloidin and paraffin according to the method of Péterfi. Sections were cut at 6μ and stained with hematoxylin and eosin.

Observations

Weights

Weights were taken once a month. If variations among individual mice in a cage exceeded 4 gm., the animals were weighed individually;

Table I

Mean Weights of Mice of the Various Groups

Type of diet	Color and sex of animals	Weights in grams at the age of months				
		3	6	9	12	15
Stock	Gray males	23.4	26.1	26.4	28.5	22.0
	Yellow males	23.6	26.3	28.1	27.6	25.0
	Gray females	17.5	20.3	21.5	22.3	22.0
	Yellow females	19.1	23.7	26.2	24.1	22.8
High-fat	Gray males	22.5	25.4	26.8	26.3	25.1
	Yellow males	25.0	30.0	35.0	28.7	25.0
	Gray females	20.3	23.0	24.5	24.9	24.0
	Yellow females	25.5	34.4	44.4	39.4	27.8
High-carbohydrate	Gray males	22.6	28.3	27.5	28.6	
	Yellow males	25.4	30.9	32.0	26.8	
	Gray females	18.0	22.5	24.0	24.0	
	Yellow females	23.5	35.5	36.7	31.8	

otherwise means were computed from the total in each cage. In Table I, the mean weights at the ages of 3, 6, 9, 12, and 15 months are given as far as mice of these ages were available at those periods.

Gray males showed uniform weight increase irrespective of the diet fed. The maximum was reached at 9 to 12 months of age; thereafter a drop was noted. The mice fed the high-carbohydrate diet weighed slightly more than those of the other groups. This may have been due to the fact that these mice were born in the spring, while only fall and winter animals comprised the other two groups.

Yellow males fed the stock diet showed about the same weight as gray males. Yellow males receiving the enriched diets were heavier than gray males fed correspondingly, the maximum difference being about 30 per cent. This increase was conspicuous during the second half of the first year of life. After the age of 1 year, the animals lost weight suddenly; at 15 months, the gray and yellow males weighed about the same.

Gray females showed uniform weights which stayed somewhat be-

low those of the gray males. Under the effect of the enriched diets, these weights increased only slightly.

Yellow females receiving the stock diet were slightly heavier than both their yellow male and gray female controls. Feeding of enriched diets caused a marked increase in weight amounting to about 80 per cent over that of the yellow females receiving the stock diet. This gain in weight was apparent at 3 months of age and rose further to the age of 9 months. Thereafter, loss of weight occurred, and at the age of 15 months the difference in weight was reduced to about what it had been at the age of 3 months, that is, about 20 per cent. Individual variations were marked. Some mice responded with little gain in weight, while others gained as much as 100 per cent, the maximum weight observed being 68 gm.

Changes in the Eyes

Gross Observations. The earliest ocular changes consisted of whitish spots or opacities in the cornea. These spots gradually increased in size, and the eyeballs became prominent (Figs. 1 and 2). Subsequently corneal ulcers developed, and a staphyloma was seen to protrude over the surface of the cornea. In many cases the lid could not be moved over the eyeball. Ultimately the eyeball shrank, showing marked distortions. The lesions usually started in one eye but sooner or later both eyes were affected.

Incidences, Sex and Age Distribution. In mice with yellow coat fed the stock diet, the earliest changes in the eyes were observed at the age of 5 months, while these lesions were not seen before the age of 10 months in mice with gray coat. The incidences observed at various ages in animals fed the various diets are given in Table II.

Of the animals fed the stock diet and about 9 months of age, only those with yellow coat had developed ocular lesions, 21.1 per cent of the females and 33.3 per cent of the males being affected. At 13 to 14 months all yellow and all gray males and 87.5 per cent of the females showed ocular changes. Gray females showed a consistently lower incidence (63 per cent), even at the mean age of 14.3 months.

Of the animals fed the fat-enriched diet and about 9 months old, the gray females had no ocular lesions; all others showed an incidence higher than that seen in the mice fed the stock diet. In gray males 45.4 per cent were affected, as compared with the 0 per cent incidence in the controls fed the stock diet. In yellow males as well as in yellow females the incidence had risen to 55 per cent as compared with 33.3 and 21.1 per cent in the corresponding mice fed the stock diet. At 12

to 14 months the incidence had increased over that found at 9 months. All yellow male mice as well as females then had lesions of the eye. Of the gray females 64.8 per cent were affected, but this percentage was not higher than that observed in corresponding females fed the stock diet. The incidence in gray males (76.2 per cent) was below that of their controls (100 per cent) fed the stock diet. However, this difference may have been due not so much to the diet as to the lower mean age of the mice of these two groups (12.8 months for the mice

TABLE II

Incidences of Ocular Lesions at Various Ages and in Various Experimental Groups

Type of diet	Color and sex of animals	No. of mice	Mean age (months)	Percentage of mice showing ocular lesions
Stock	Gray males	10	9.6	0.0
	Yellow males	12	8.9	33 3
	Gray females	17	9.1	0.0
	Yellow females	19	9.1	21.1
	Gray mlaes	19	14.1	100.0
	Yellow males	24	13.5	100.0
	Gray females	27	14.3	63.0
	Yellow females	32	13.6	87.5
High-fat	Gray males	11	9.4	45.4
	Yellow males	20	8.7	55.0
	Gray females	18	9.0	0.0
	Yellow females	20	8.7	55.0
	Gray males	21	12.8	76.2
	Yellow males	14	12.6	100.0
	Gray females	17	14.2	64.8
	Yellow females	26	14.3	100.0
High-carbohydrate	Gray males	22	10.2	45.5
	Yellow males	11	10.3	91.9
	Gray females	19	9.3	26.3
	Yellow females	20	10.0	80.0

fed the high-fat diet and 14.1 months for the mice fed the stock diet).

Of the animals receiving the high-carbohydrate diet and 9 to 10 months of age, the yellow-coated individuals were particularly susceptible, 91.1 per cent of the males and 80 per cent of the females showing ocular lesions. Gray females with an incidence of 26.3 per cent were least affected. (This percentage may have to be corrected because the animals were, on the average, 1 month younger than those of the other groups. The mice in this series are not old enough at present to give data regarding the ultimate development of the disease. As far as the onset of the ocular lesions is concerned, the age of 10 months seemed to be critical.)

Microscopic Examination. The early changes consisted of thicken-

ing and keratinization of the corneal epithelium, and in many instances pearls of keratin could be identified. As the lesions increased in severity, many epithelial cells underwent swelling or ballooning with the formation of small cyst-like spaces. Bowman's membrane was destroyed, and the cornea became infiltrated with polymorphonuclear and mononuclear leukocytes. The epithelium underwent necrosis and was sloughed off; ulcers formed. The corneal epithelium adjacent to the ulcers proliferated mitotically, but increasing numbers of polymorphonuclear and mononuclear leukocytes interfered with the process of regeneration. Subsequently, Descemet's membrane was destroyed, and the inflammatory process extended into the anterior chamber of the eye and into the eyelids. Granulation tissue formed and led to anterior synechia of the iris, and spread through the defect of the cornea. It also extended between chorioidea and retina, and detachment of the latter resulted. Some of the findings are illustrated in Figures 3 to 7.

During the early stages, no pathogenic organisms were found in the conjunctival sac. In the advanced stages, some diplococci and a few pneumococci were identified. These organisms probably should be considered secondary invaders without pathogenic significance.

Discussion

Keratomalacia followed by keratitis, iridocyclitis, and panophthalmitis was observed in mice of strain YBR/Wi. Both gray-coated and yellow-coated individuals were affected. Animals with yellow coat color and males developed these changes at an earlier age than gray-coated individuals or females. Of all animals examined, gray-coated females seemed to be most resistant. Diets enriched by fat or carbohydrate tended to increase the frequency of the ocular disease, at least at an early age.

The pathogenesis of the keratomalacia is unknown, and at this time only tentative explanations can be offered as to its nature. A similarity of the changes observed to those described in vitamin A deficiency[11,12] is striking, and the occurrence of skin lesions in yellow mice[3] may likewise be explained on the basis of vitamin A deficiency.

Our diets have been found to be adequate in vitamin A for other strains of mice.[8,9] Therefore, one may have to assume that mice of the yellow strains differ from those of other strains as far as requirements for vitamin A are concerned. Such a strain difference would not be unusual, since the requirements for other vitamins have been shown

to differ in various strains of mice.[13] In view of the latent or overt diabetes present in yellow mice, there is also the possibility of a disturbance in the intermediary metabolism of vitamin A. Human diabetic patients suffer from an inability to convert carotene into vitamin A,[14,15] and a similar defect may be present in yellow mice. Since the supposedly non-diabetic gray-coated individuals likewise developed keratomalacia, the susceptibility to the latter may not be related to the diabetic constitution of the individual as such. This seems to be likely because the ocular lesions observed by us bore no resemblance to those frequently associated with human diabetes, which, commonly, are not attributed to the disturbance in the metabolism of vitamin A.

Investigations are in progress to ascertain the rôle of vitamin A in the pathogenesis of the ocular disease described. The possibility of other etiologic factors, however, cannot be ruled out.

Summary

Keratomalacia, followed by keratitis, iridocyclitis, and panophthalmitis, was observed in mice of strain YBR/Wi. Both gray-coated and yellow-coated individuals were affected. Males were more susceptible to the disease than females. Animals with yellow coat color developed these ocular changes at an earlier age than gray-coated individuals. Diets enriched in fat or carbohydrate tended to accelerate the appearance of the ocular lesions.

We are indebted to Dr. Louis Yuan for the photographs.

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[*Illustrations follow*]

LEGENDS FOR FIGURES

FIG. 1. Early corneal lesion in a gray male mouse, 10 months old.

FIG. 2. Diffuse keratomalacia in a yellow female mouse, 12 months old.

FIG. 3. Cross section through the eyeball of a yellow male, 11 months old, showing thickening of the corneal epithelium and diffuse keratitis. × 45.

FIG. 4. Part of Figure 3 showing squamous metaplasia of the corneal epithelium and destruction of Descemet's membrane with anterior synechia. × 200.

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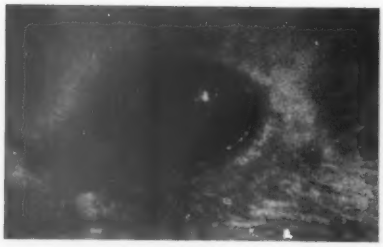

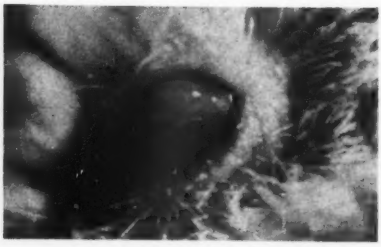

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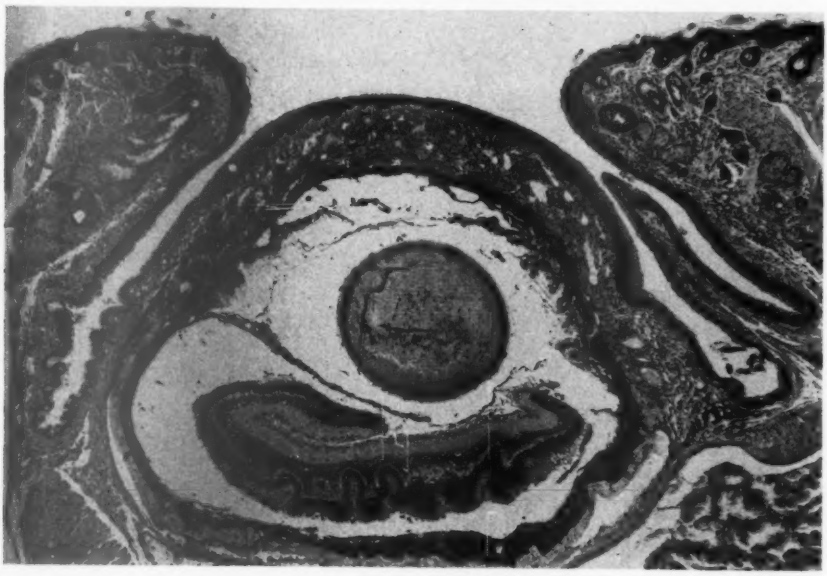

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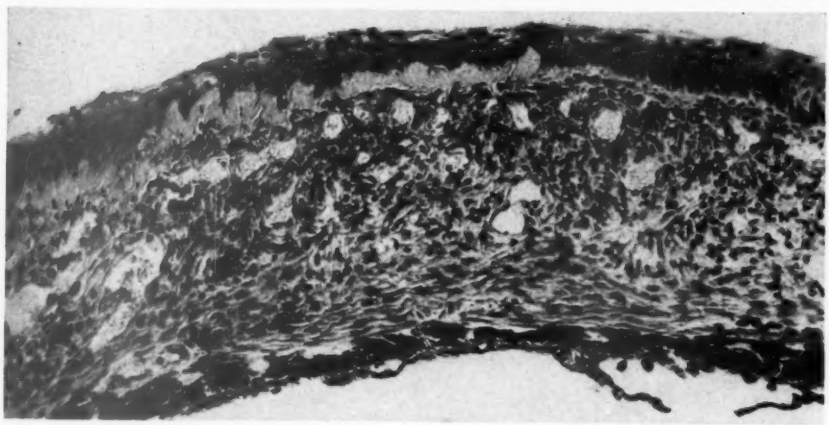

FIG. 5. Section through the cornea of a yellow male, 7 months old (early change). There are ballooning, vesiculation, and squamous metaplasia of the corneal epithelium. × 400.

FIG. 6. Part of section through the eye of a gray male, 11 months old. Squamous epithelium of conjunctival sac at upper right, lacrymal glands at lower right. There is thickening of the chorioidea and formation of granulation tissue in the chorioidea. Finely dispersed pigment may be seen in the knob of granulation tissue that lifts the retina from the chorioidea. × 150.

FIG. 7. Section through the cornea of a yellow male, 8 months old, showing ulcerative keratitis with squamous metaplasia of the surviving epithelium. × 200.

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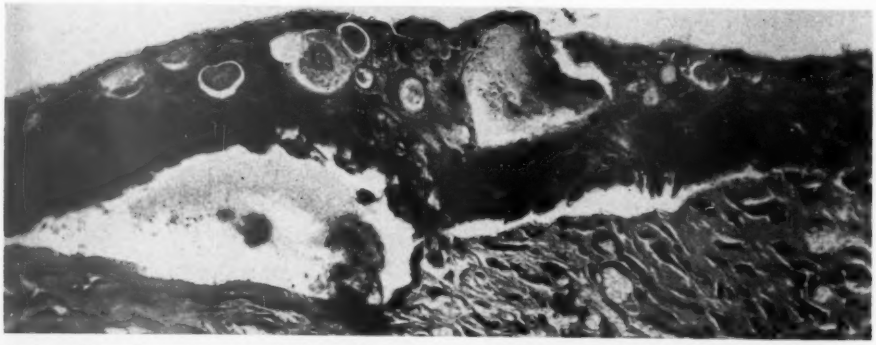

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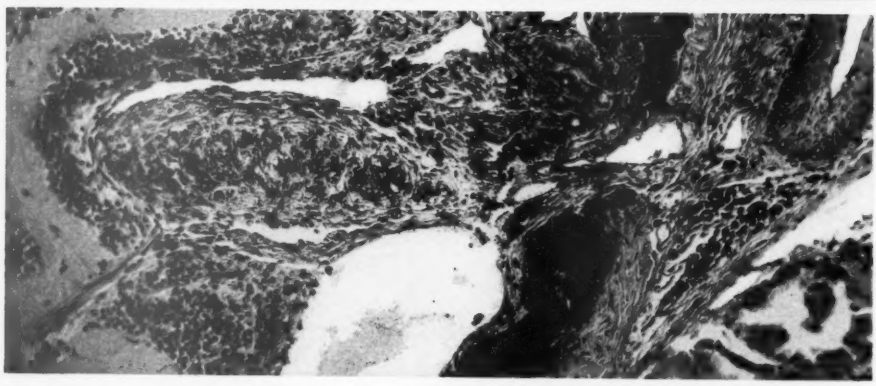

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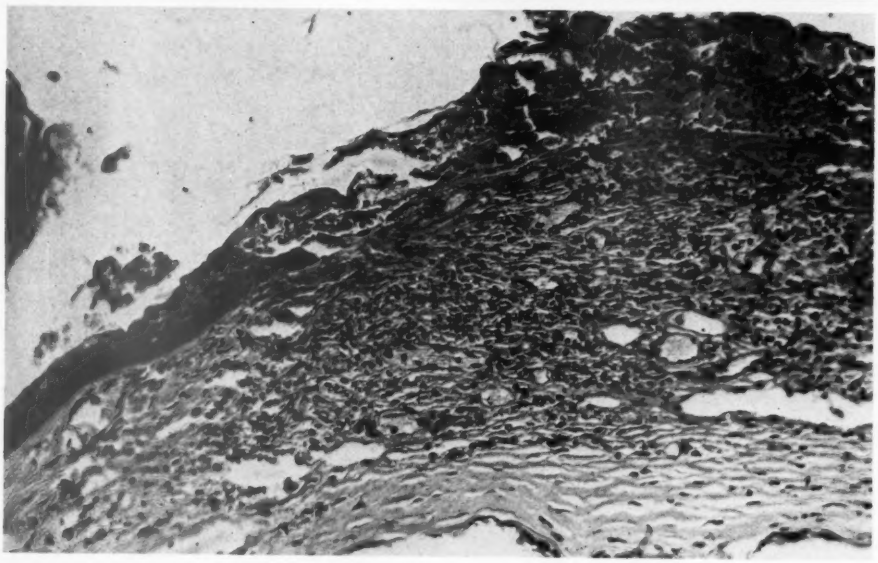

7

THE ABSORPTION OF COLLAGEN IN THE LIVER

I. Histologic Changes Accompanying the Absorption of Implanted Surgical Gut in the Liver *

Henry Ungar, M.D., and Joseph D. Feldman, M.D.

(From the Department of Pathology of the Hadassah University Hospital and the Hebrew University-Hadassah Medical School, Jerusalem, Israel)

A method of studying the reaction of fibrous tissue and the disposition of collagen implanted into the liver was reported previously.[1]

With this method a thread of homologous rat tendon or plain surgical gut (sheep collagen) was fixed in a lobe of the liver. Homologous tendon remained intact but stimulated the development of a narrow capsule of vascularized newly formed connective tissue which persisted for at least 2 months. Surgical gut, however, was absorbed within 3 weeks and stimulated the proliferation of collagenous connective tissue, which regressed leaving a minute scar.

The technique of implantation offers several advantages in the study of regression of hepatic cirrhosis which has been suggested by the observations of several authors.[2-7] First, the quantity of collagen presented to the liver for absorption can be controlled. Second, there is a single quantitative stimulus for the formation of reactive fibrous tissue by the liver. Lastly, the temporal sequence of events can be accurately ascertained.

The purpose of the present paper is to describe the histologic changes which accompany the absorption of surgical gut implanted in the liver in animals maintained on a balanced diet, as a preliminary step to an investigation of the various nutritional, endocrine, and other factors which may influence the regression of fibrosis.

Methods

Eighteen white male rats, ranging in initial weight from 180 to 250 gm., were kept on a standard stock diet. A laparotomy was performed, and a thread of plain surgical gut, size 3/0, heat sterilized and preserved in iodized alcohol by the manufacturers, was transversely implanted through the large left lobe of the liver. The animals were sacrificed in pairs following the operation as follows: at 2 and 4 days; 1, 2, and 3 weeks; 1 and 2 months. Four animals were examined at

* Received for publication, March 31, 1953.

the 3-week and 1-month periods. Fragments of the operated lobe were fixed in Zenker's fluid. Paraffin sections at 8 μ were stained with hematoxylin and eosin, and by Laidlaw's silver method, counterstained with van Gieson's stain.

Results

Initially there was a narrow zone of necrotic liver tissue which surrounded the implanted collagen gut. In this zone the reticular fibers of the hepatic parenchyma were preserved in a normal architectural arrangement, and there was no evidence of reticulum collapse (Fig. 1). The implanted gut underwent a series of alterations similar to those associated with the presence of foreign material in living tissue. The immediate reaction consisted of an accumulation of neutrophils around the collagen. It was accompanied by a fibrous tissue reaction and by the growth of a rich capillary bed and numerous histiocytes.

Within 4 days there was a marked increase of fibrous tissue, represented by numerous young fibroblasts arranged in a circular pattern and by a moderate number of argyrophilic fibers adjacent and parallel to the surface of the fibroblasts (Figs. 2 and 3). A few of these argyrophilic fibers were branched and tortuous. Mitotic figures were seen occasionally in fibroblastic elements. Within 1 week the connective tissue cells and argyrophilic fibers increased to form two roughly spherical nodules of dense fibrous tissue (Fig. 4). During the second week the fibrous tissue appeared to mature. The fibroblastic nuclei decreased in number and became flattened and darker (Fig. 5). The argyrophilic fibers thickened into dense concentric layers (Fig. 6). During this period the capillaries, neutrophils, and histiocytes had cleaved the implanted material, and within 2 weeks numerous typical foreign body giant cells had appeared along the edges of the split collagen (Fig. 5).

Beyond this period there was a slow regression of the reacting fibrous tissue. There was an actual diminution of fibroblasts and argyrophilic fibers. The spherical form of the surrounding fibrous tissue was altered so that small bands of mature fibrous tissue formed septa and divided the lesion into several round areas. At 3 weeks the collagen was almost completely disintegrated, and fragments of birefringent and argyrophilic fibers were found within giant cells. The process of collagen disintegration continued up to 1 month (Fig. 7), and beyond this time all remnants of the implanted collagen and all giant cells had disappeared.

After 2 months only minute band-like scars were visible, composed

of loose collagen fibers with slight infiltrations of lymphoid cells, and surrounded at the periphery by a scattering of fine argyrophilic fibrils (Fig. 8).

Discussion

The experimental procedure permitted observations on the temporal development and regression of hepatic fibrous tissue. New collagen fibers were seen on the fourth day, and they increased to a peak by the end of 1 week after implantation of foreign collagen. During the following week the fibrous tissue seemed to mature and contract slightly. Thereafter there was a gradual diminution of collagen until, at 2 months, only a small, irregular scar of loose acellular connective tissue remained.

Absorption of the surgical gut took place in the liver as would regularly be expected in other tissues of the body. Since, in addition, surgical gut is composed of heterologous collagen, denatured by heat sterilization and preserved in iodized alcohol, we do not consider a discussion of this part of the observation within the scope of the present experiment.

The stimulus for fibrous tissue synthesis probably was derived either from the breakdown products of the foreign collagen or from the products of the initial neutrophilic infiltration. What these products might be was not determined in the present experiments. Also, the possibility was not excluded that the antiseptic preservative of the surgical gut may have caused the fibrous tissue reaction.

The new production of collagen fibers was most likely not consequent to necrosis of hepatic cells or to hemorrhage. A narrow zone of necrotic hepatic tissue was seen 2 days after implantation but not beyond that time. The most marked production of collagen occurred between the fourth and seventh days, after all signs of necrosis had disappeared. Hemorrhage around the implanted collagen was minimal and had disappeared before fibrous tissue production began. Similarly, we have observed only insignificant fibrosis consequent to the trauma caused by implantation into the liver of homologous tendon having approximately the same caliber as the gut.[1]

Several investigators maintained that a reversal of experimental fibrosis in the liver may be simulated by the condensation or stretching of fibrous trabeculae following increased regeneration of the hepatic parenchyma as a result of improved metabolic conditions.[5,8] Parenchymal regeneration was not seen in the present material, and the sequence of proliferation followed by regression of the fibrous tis-

sue seems to indicate an actual absorption of collagen, corroborating the observations of Morrione,[7] who found (by quantitative chemical methods) decreasing amounts of collagen during the period when "cirrhogenic" treatment of animals was stopped. In the light of the histologic events described here, regression is best explained as a result of enzymatic dissolution of fibers with absorption into tissue fluids, as suggested by the work of Rössle[9] and Gersh and Catchpole.[10] The same authors have questioned whether epithelial or mesenchymal elements secrete collagenases in explaining the "labile" state of collagen in the organism. The data of this and previous experiments do not permit us to decide this question. However, it seemed most likely that the early leukocytic infiltration did not provide the enzymes for the process of resorption.

Summary

Collagen in the form of surgical gut, implanted into the liver, stimulates the production of new fibrous tissue. Fibroblasts and argyrophilic fibers increase to a maximum for a period of 7 days. These elements mature for about 1 week and then slowly disappear. Two months after implantation only a small, loose scar of connective tissue remains.

The implanted collagen is fragmented and completely resorbed within 1 month. This is accomplished by the combined action of new capillaries and of histiocytes and giant cells.

Dr. Zvi Neuman performed the operations reported in this article.

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[*Illustrations follow*]

LEGENDS FOR FIGURES

FIG. 1. Two days following implantation, the surgical gut was surrounded by abundant neutrophilic leukocytes and a clearly defined zone of necrotic parenchyma. Laidlaw-van Gieson's stain. × 145.

FIG. 2. Abundant fibroblasts are shown surrounding the gut, 4 days following implantation. Scattered mitotic figures are present. Hematoxylin and eosin stain. × 510.

FIG. 3. Same area as shown in Figures 1 and 2, showing beginning formation of reticulum fibers. Laidlaw-van Gieson's stain. × 510.

FIG. 4. At 1 week following implantation, a wide zone of concentric reticulum fibers surrounds the gut. Laidlaw-van Gieson's stain. × 145.

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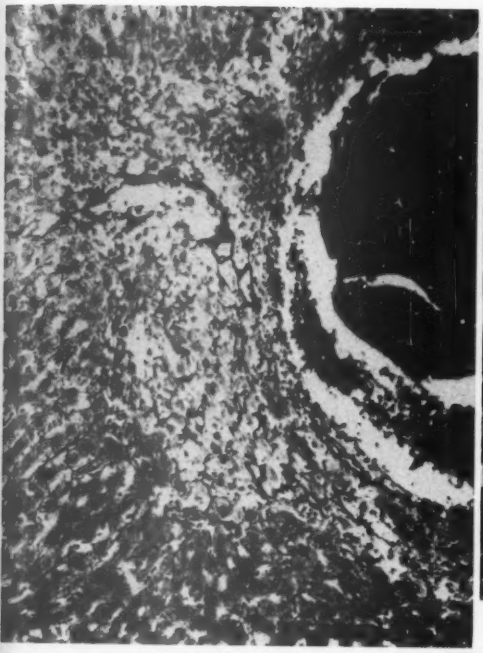

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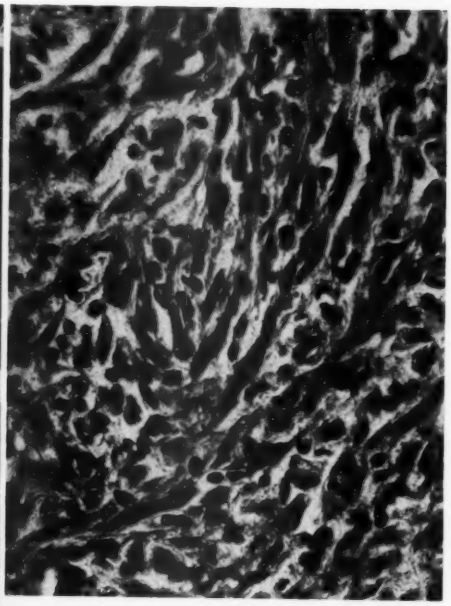

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FIG. 5. After 2 weeks, the fibrous ring around the gut has decreased in width, and correspondingly the number of fibrocytes is considerably less than at the earlier period. Hematoxylin and eosin stain. × 140.

FIG. 6. Same area as in Figure 5. There is evidence of a decrease in reticulum fibers as compared with 1 week earlier (see Fig. 4). Laidlaw-van Gieson's stain. × 140.

FIG. 7. After 1 month, the focus contains only sparse reticulum fibers, and no collagen areas appear to be present in the center. Laidlaw-van Gieson's stain. × 140.

FIG. 8. At 2 months following the operation, only a small, loose scar remains, showing moderate residual inflammation. Hematoxylin and eosin stain. × 140.

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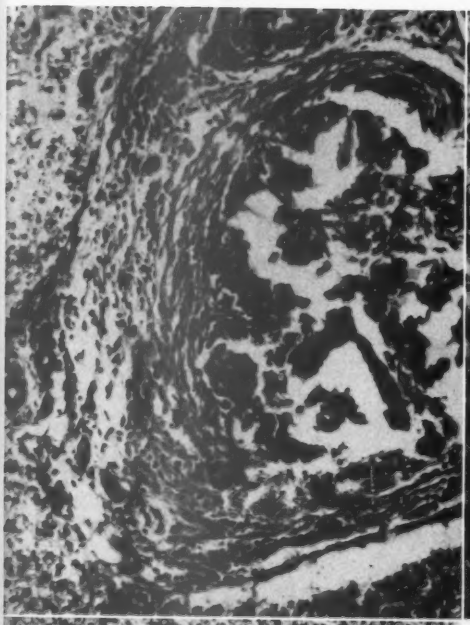

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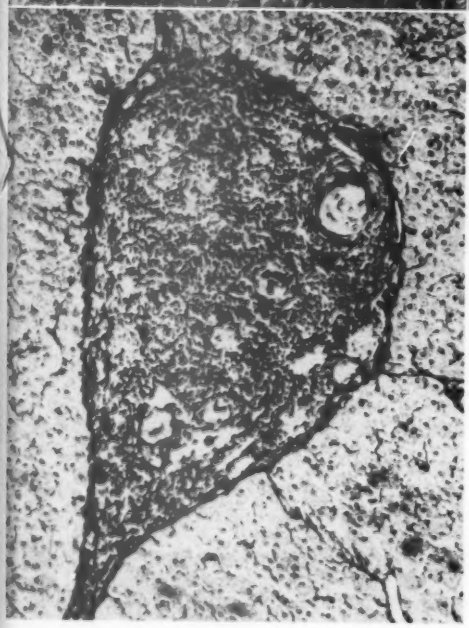

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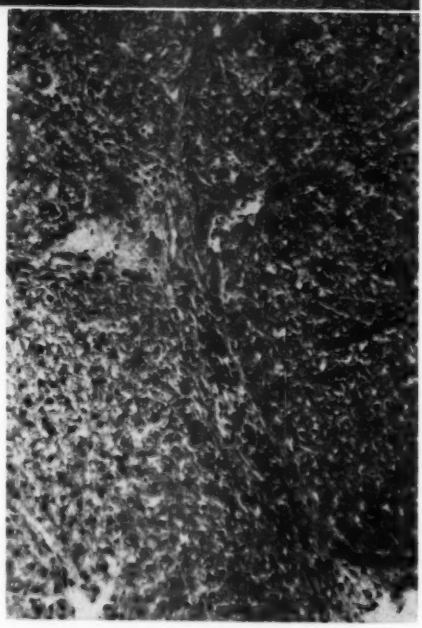

8

THE ABSORPTION OF COLLAGEN IN THE LIVER

II. Observations on the Absorption of Implanted Surgical Gut Under Various Dietary Conditions *

Henry Ungar, M.D.

(From the Department of Pathology of the Hadassah University Hospital and the Hebrew University-Hadassah Medical School, Jerusalem, Israel)

In a previous paper it was reported that foreign collagen, in the form of surgical gut in the livers of rats, stimulated at first the new formation and later the reabsorption of fibrous tissue. These processes were seen to devolve during regular intervals of time.[1,2]

The purpose of the experiments presented in this article was to investigate the influence of variations in the dietary components on the fate of the implanted collagen and the reactive fibrosis in the liver. It was expected to throw light on some of the controversial points arising from experiments dealing with the dietary treatment of hepatic cirrhosis which were reviewed recently by Plough, Patek, and Bevans.[3]

Material and Methods

Sixty-four male white rats, ranging in initial weight from 180 to 250 gm., were divided into four groups and maintained on the diets shown in Table I. The diets were adapted from those used by Morrione[4] and György and Goldblatt[5] in accordance with the local availability of the components. The animals were placed on the diets 2 weeks before a piece of plain surgical gut (sheep collagen), size 3/0, was introduced into the large left lobe of the liver, as previously described.[2] After the operations the animals were sacrificed in pairs as follows: at 2 and 4 days; 1, 2, 3, and 4 weeks; and 2 months. Fragments of the operated lobes of the livers were fixed in Zenker's fluid. Paraffin sections at 8 μ were stained with hematoxylin and eosin, Laidlaw's silver method counterstained with van Gieson's, Weigert's elastic tissue stain, Gomori's method for hemosiderin, Ciaccio's Sudan solution and Ziehl-Neelsen's method.

Results

Morphologic changes surrounding the implanted surgical gut up to about 2 weeks following the operation were similar to those previously reported in animals maintained on a balanced diet.[2] Following early

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Presented at the Fiftieth Annual Meeting of the American Association of Pathologists and Bacteriologists, St. Louis, April 2, 1953.

necrosis, within 4 days a ring of granulation tissue had formed, containing abundant fibroblasts and showing beginning formation of reticulum fibrils, parallel and adjacent to the fibroblasts. Abundant neutrophilic leukocytes were seen near the gut in all animals except those receiving a high fat diet; in the latter, leukocytes were scarce or entirely absent from the exudate.

At the end of 1 week the implanted thread was invaded by minute capillaries and surrounded by foreign body giant cells. In the peripheral zone of the granulation tissue fibroblasts were now more predominant, but in the animals with diffuse fatty change of the parenchyma the reactive zone was distinctly narrower than in the other series. Abundant reticulum fibrils were present in rats maintained on a high carbohydrate-low fat diet, precisely as seen in rats on a balanced diet in the previous experiments. In all animals of the low protein-moderately high fat series the deposition of dense collagen material was more prominent, revealing only a small amount of argyrophilic fibrils. This finding remained the same during the second and third weeks.

Two weeks following implantation the gut was surrounded by numerous foreign body giant cells, penetrated by narrow capillaries and reduced in size. The fibrous tissue surrounding the gut was present in about equal quantity in all animals irrespective of their diet, but with certain qualitative differences: the argyrophilic fibrils were as scarce as they had been at the end of the first week in the animals on the low protein-moderately high fat diet (Fig. 1); only in the animals on the high fat (40 per cent) and the high carbohydrate-low fat diets did signs of inflammation still appear active (Figs. 2 and 3).

No significant additional changes developed during the third week except that the gut was even more reduced in rats receiving low protein diets with moderately high amounts of fat.

At the end of 1 month the differences in the appearance of the foci in the various series were more pronounced than at any other period of observation. The gut was found either in minute quantity or entirely absent in the rats on the low protein diet, whether or not they had received supplements of lipotropic factors (Figs. 4 and 5). In the same series dense scars had formed containing scarce nuclei, a few giant cells, and accumulations of macrophages containing ceroid-like material or hemosiderin granules. The gut appeared to be slightly better preserved in the high carbohydrate group. Here, as well as in the high fat (40 per cent) series, reticulum fibrils now appeared more abundant than before (Figs. 6 and 7). The lesions in the rats main-

tained on a high fat (40 per cent) diet showed little change, and the implanted gut revealed no further decrease in size (Fig. 6).

Two months following implantation (about 75 days after instituting the various diets) the lesions were reduced to minute, loose scars in all animals except those fed the high fat (40 per cent) diet (Figs. 9, 10, and 11). In addition, the livers of the rats on the low protein-moderately high fat diet contained numerous fat cysts but no visible increase

TABLE I

Composition of Diets *

	Low protein-moderately high fat	Low protein-moderately high fat†	Low protein-high fat	High carbohydrate-low fat
	%	%	%	%
Casein	8	8	8	18
Cornstarch	66	66	48	74
Lard	10	10	19	
Cocosin‡	10	10	19	2
Cod liver oil	2	2	2	2
Salt mixture no. 2, U.S.P.	4	4	4	4

* To every kilogram of diet the following were added: thiamine hydrochloride, 10 mg.; riboflavin, 20 mg.; pyridoxine, 10 mg.; calcium pantothenate, 500 mg.; niacin, 100 mg. Each animal received daily supplements of 20 μg. of vitamin K (menadione).

† Supplemented with DL-methionine, 500 mg.; choline chloride, 125 mg.; and L-cystine, 312.5 mg./100 gm. of diet.

‡ A cocoanut oil margarine, manufactured by the Blue-Band Margarine Co., Haifa, Israel.

in fibrous tissue (Fig. 8). In the high fat (40 per cent) series the gut was slightly reduced in size and surrounded by a moderately wide capsule of reticular fibrous tissue (Fig. 12).

Throughout the experimental period no elastic fibrous tissue was demonstrated in the lesions outside the walls of blood vessels.

DISCUSSION

In a preceding article it was shown that implantation of surgical gut in the liver of rats maintained on a balanced diet stimulated a marked perifocal fibrosis. This reached a peak about 1 to 2 weeks following the operation and later regressed, leaving a small scar after about 2 months. By the latter half of the first month complete absorption of the implanted gut had taken place.

The experiments revealed that the livers of animals maintained on a low protein-moderately high fat diet (known eventually to produce

diffuse cirrhosis) or on a high carbohydrate-low fat diet did not lose the capacity to absorb fibrous tissue stimulated by a foreign body reaction. There was no difference in reaction when the diet was supplemented by a mixture of lipotropic factors.

These findings seem to support earlier observations regarding the spontaneous regression of early experimental cirrhosis[4,5] and suggest that the progressive stages of cirrhosis may depend not as much on the primary vitality of the hepatic parenchyma[7] as on secondary changes in the vasculature[8,9] or on extrahepatic metabolic or possibly hormonal factors.[5]

Absorption of the introduced foreign material (sheep collagen) and of reactive fibrosis was absent in animals fed a low protein diet with an extremely high fat (40 per cent) content. One might assume that the absorption of the surgical gut and the reactive fibrous tissue was interfered with by the extreme fatty change in the liver cells (which, in the opinion of several investigators, stimulates fibrosis mechanically[10]) or was a result of ischemia subsequent to compression of the sinusoids by the infiltrated cells.[11] However, these views were not borne out by my findings that fatty change of similar degree in the livers of rats on a low protein-moderately high fat diet caused no delay in the absorption of the collagen.

The arrest of absorption in rats on the high fat (40 per cent) diet may be due to the absence of initial leukocytosis as a preliminary to the dissolution of the foreign collagen or to the presence of a greater quantity of non-saturated fatty acids in the lard, which has been shown to be detrimental to the course of experimental cirrhosis.[5,12] There exists, however, the possibility that in the experiments reported here the process of absorption was delayed owing to anorexia and inanition which developed after 2 months in all rats fed the high fat (40 per cent) diet.

No comment can be made at present regarding the development of dense, firm, collagen tissue in rats on the low protein diets as compared with the abundance of loose argyrophilic reticulum surrounding the implanted gut in rats on a balanced diet and on a high carbohydrate-low fat diet. However, these quantitative differences in the composition of the reactive fibrous tissue appeared unrelated to its final absorption.

Summary

Morphologic changes were studied following implantation of plain surgical gut in the livers of rats maintained on various diets. The previously described fibroblastic reaction around the gut in animals on a balanced diet became modified as follows:

Within about 3 weeks the implanted gut in the livers of rats on the low protein-moderately high fat diet was absorbed. The same occurred in rats on a high carbohydrate-low fat diet during the second month. In rats on the low protein-high fat diet the gut remained unabsorbed during a 2-month observation period.

Regression of newly formed fibrous tissue was observed in animals on a low protein-moderately high fat diet and a high carbohydrate-low fat diet.

Addition of lipotropic substances to the low protein-moderately high fat diet did not prevent the new formation, or accelerate the absorption, of fibrous tissue.

I wish to express my appreciation to Dr. James F. Rinehart, Professor of Pathology at the University of California School of Medicine for the use of laboratory facilities during completion of this paper.

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[*Illustrations follow*]

LEGENDS FOR FIGURES

FIG. 1. Two weeks following implantation of surgical gut. Low protein-moderately high fat diet. The gut is attacked by a foreign body reaction and surrounded by a ring of dense collagen tissue. Hematoxylin and eosin stain. × 130.

FIG. 2. Two weeks following implantation of surgical gut. Low protein-high fat (40 per cent) diet. Foreign body reaction as in Figure 1, but the new formation of reactive fibrous tissue appears delayed. Hematoxylin and eosin stain. × 130.

FIG. 3. Two weeks following implantation of surgical gut. High carbohydrate-low fat diet. Active foreign body reaction. New formation of dense fibrous tissue is conspicuous. Hematoxylin and eosin stain. × 130.

FIG. 4. Four weeks following introduction of surgical gut. Low protein-moderately high fat diet. Diffuse fatty change of liver cells. The gut is completely absorbed and replaced by firm, dense, fibrous tissue containing accumulations of macrophages with pigment. Hematoxylin and eosion stain. × 130.

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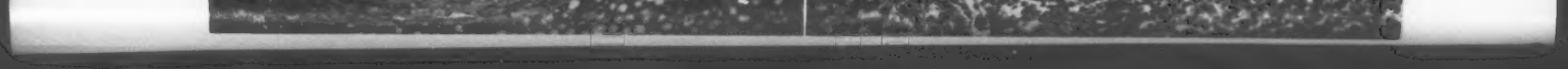

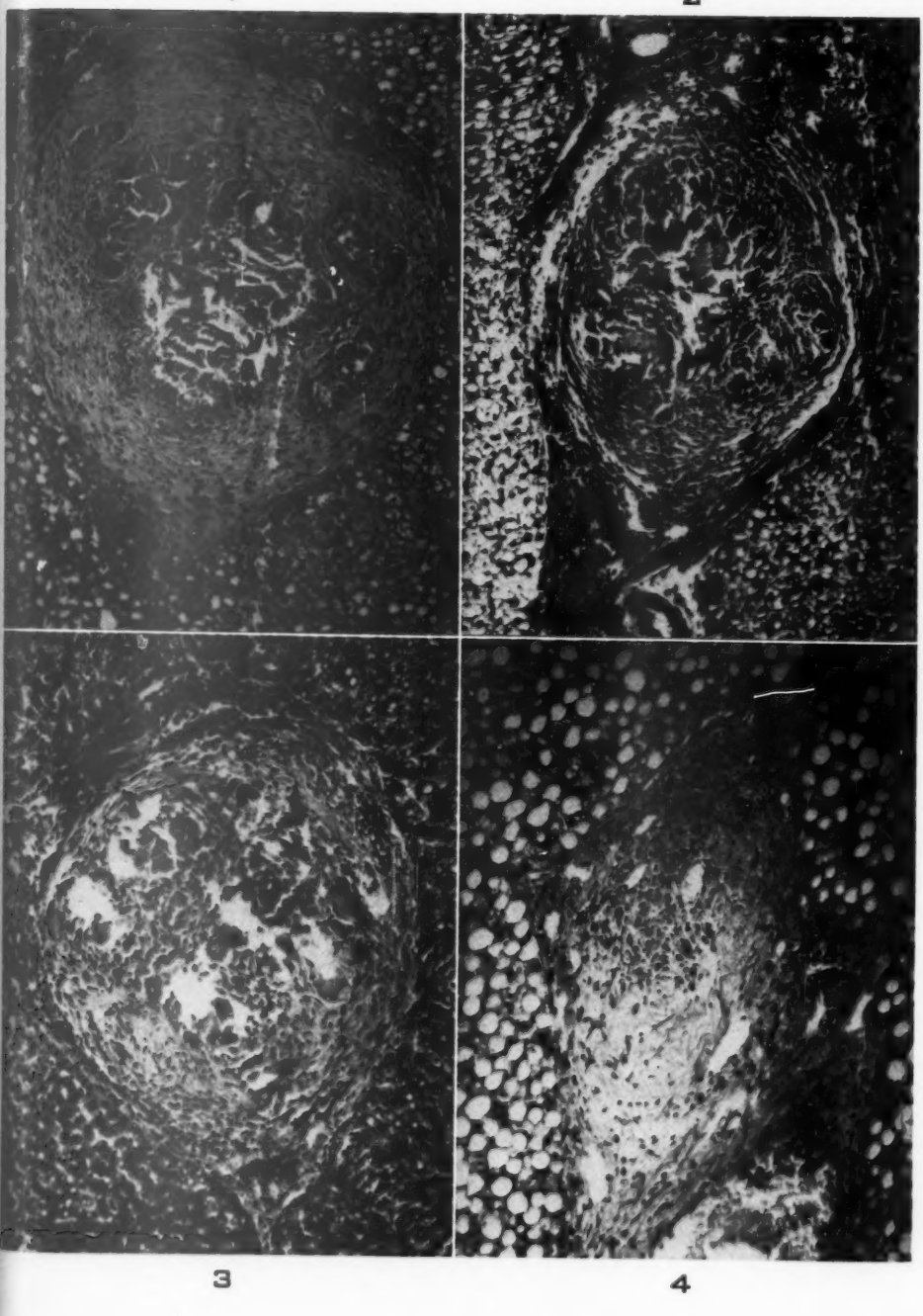
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FIG. 5. Four weeks following introduction of surgical gut. Low protein-moderately high fat diet supplemented by DL-methionine, choline, and L-cystine mixture. Fibrous lesion similar to that shown in Figure 4. The giant cells contain remnants of animal hair inadvertently introduced during the operation. Hematoxylin and eosin stain. × 130.

FIG. 6. Four weeks following introduction of surgical gut. Low protein-high fat (40 per cent) diet. Delay in the absorption of gut. The foreign material is surrounded by a few giant cells and dense, fibrous tissue. Laidlaw-van Gieson's stain. × 130.

FIG. 7. Four weeks following introduction of surgical gut. High carbohydrate-low fat diet. Delayed absorption of the implanted gut. Laidlaw-van Gieson's stain. × 130.

FIG. 8. Two months following implantation of surgical gut in liver (75 days after institution of diets). Low protein-moderately high fat diet. Diffuse fatty change and presence of fat cysts. There is no visible increase of diffuse fibrous tissue. Hematoxylin and eosin stain. × 380.

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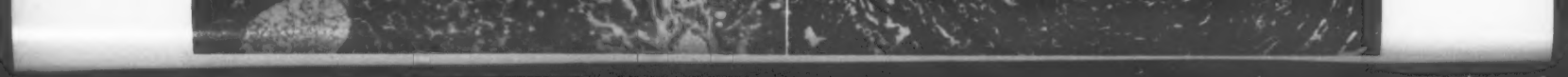

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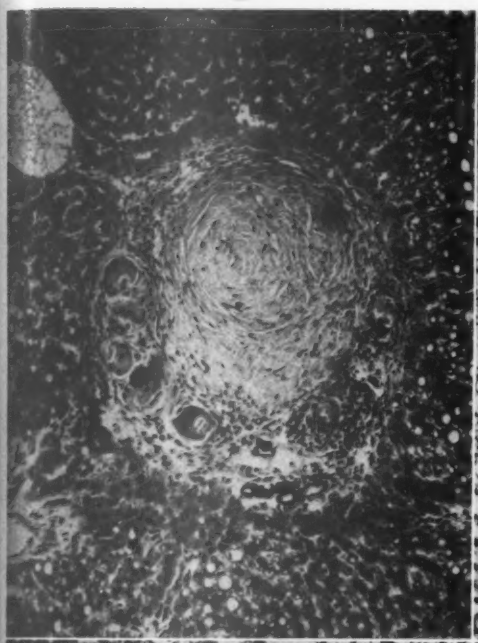

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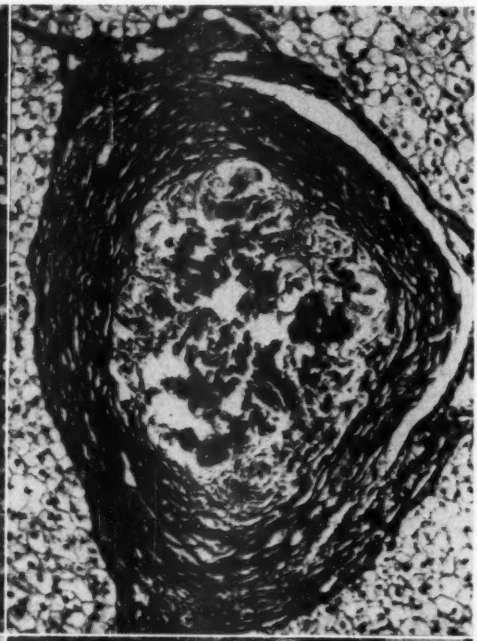

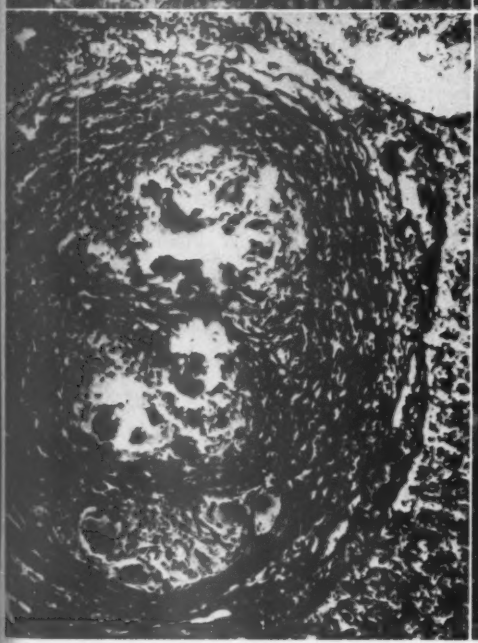

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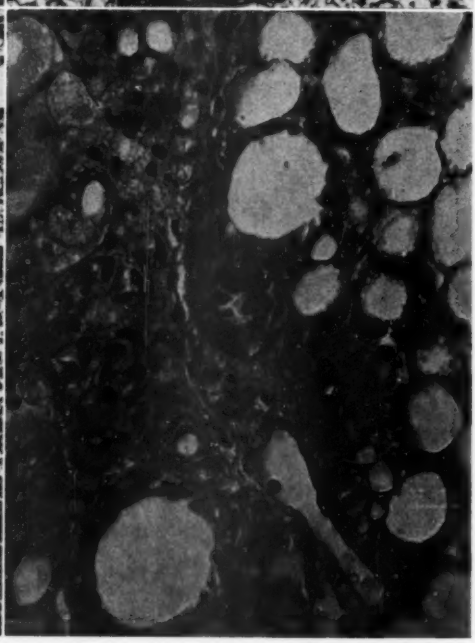

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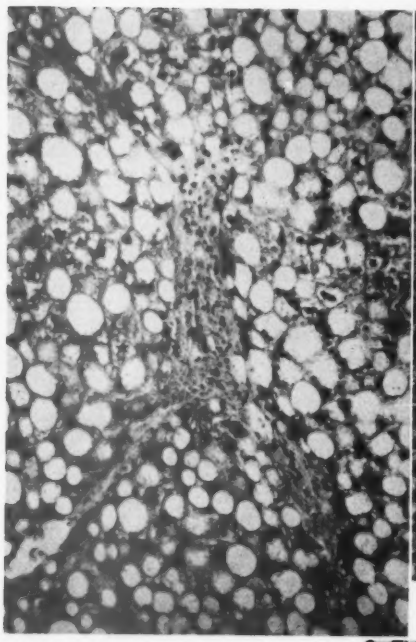
FIG. 9. Two months following implantation of surgical gut in liver (75 days after institution of diets). Low protein-moderately high fat diet. A small scar has formed at the site of implantation of the surgical gut, comparable in size to that seen in animals maintained on a balanced diet. Hematoxylin and eosin stain. × 150.

FIG. 10. Two months following implantation of surgical gut to liver (75 days after institution of diets). Same diet as indicated in legends for Figures 8 and 9 but supplemented by DL-methionine, choline, and L-cystine. Small scar at the site of implantation of surgical gut. Hematoxylin and eosin stain. × 160.

FIG. 11. Two months following implantation of surgical gut in liver (75 days after institution of diets). High carbohydrate-low fat diet. A small scar is present which contains accumulations of lymphoid cells and a few macrophages with ceroid-like pigment. Hematoxylin and eosin stain. × 150.

FIG. 12. Two months following implantation of surgical gut in liver (75 days after institution of diets). Low protein-high fat (40 per cent) diet. The implanted gut and the reactive connective tissue have remained. Laidlaw-van Gieson's stain. × 150.



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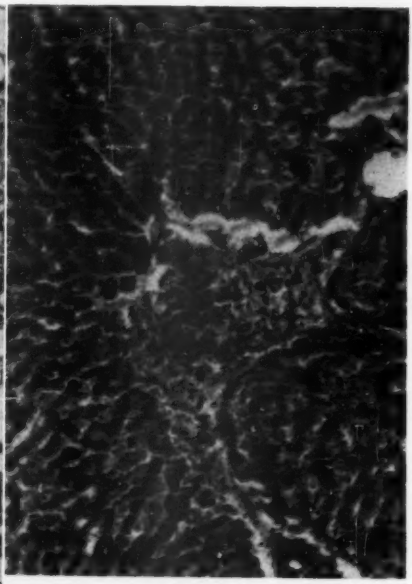

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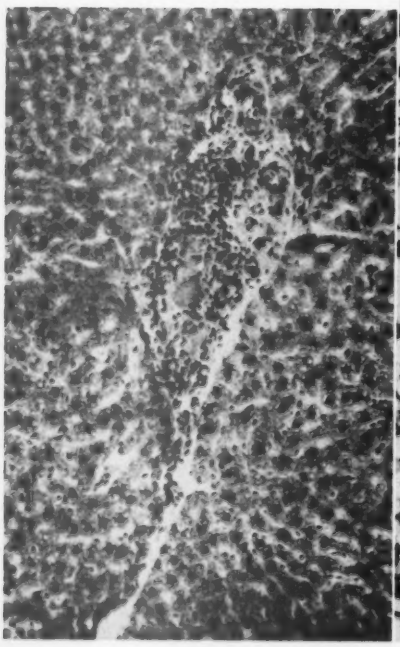

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PARACALYCEAL CYSTS OF THE RENAL SINUS *

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In the last 1100 necropsies at the Banting Institute, multiple cysts due to regressive changes were found fourteen times in the adipose tissue of the renal sinus. With increasing awareness of their appearance and genesis, these cysts are now being found to have a higher incidence than is indicated by these figures. Search of the literature has failed to disclose any previous mention of them except possibly by Rivalta[1] and he considered the cysts described by him to be due to obstruction of lymphatics.

Character of Cysts

Many of the cases showed cysts in varying stages of development and Figures 1 to 3 illustrate some of those present in case 14. The diameter ranged from a few millimeters to several centimeters. The smaller ones were ovoid and the larger were irregular, excavating the fatty tissue around the calyces and sometimes, as shown in Figure 4 (case 1), leaving the latter deprived of mesenchymal support on every side. They were always multiple and in 7 cases bilateral. In the unilateral cases, it often was noticed that the cysts were present near the kidney containing the more fat. They produced no compression of surrounding tissues and gave the impression that they were replacing and not displacing adipose tissue. When discovered, most of them had been cut and were empty, but the few seen on fresh section contained clear fluid which, under the microscope, often contained abundant oil droplets. Their lining was uniformly delicate, thin, and gray, and underneath it golden granules of hemosiderin commonly were visible. Cysts were most common near the poles of the kidneys.

The main features of these 14 cases are summarized in Table I. There was no predilection as to sex. All of the patients but one were over 60 years old and the average age was 69. No clue could be derived from the nature or duration of the terminal illnesses. Active pyogenic inflammation of the kidney and pelvis was present in only 2 cases; 4 patients were described as obese, 5 as emaciated, 3 as moderately wasted, and 2 as normal. A feature common to all was the presence of an excessive amount of fat in the renal sinuses and in 9 cases this adiposity was associated with considerable atrophy of the renal

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TABLE I

Data on Fourteen Cases of Paracalyceal Cysts

Case no.	Sex	Age	Cause of death	Edema, O Effusion, E	State of body fat	Number of cysts	Renal disease	Weight of kidneys	Fat in sinus	Regressive changes in fat	Occlusion of arterioles
(1) 319/50	M	80	Perforated gastric ulcer	— —	Moderate wasting	Multiple	Senile atrophy	gm. L. 130 R. 160	++	Slow necrosis	Patchy, complete
(2) 338/51	M	85	Cancer of esophagus	— —	Wasted	Bilateral, multiple	Senile atrophy	L. 165 R. 155	++	Slow necrosis	Patchy, slight
(3) 46/51	F	71	Cardiac infarction	— —	Obese	Bilateral, multiple	None	L. 175 R. 100	Replaced by cysts	Slow necrosis	Many, complete
(4) 350/51	M	69	Cancer of right adrenal	— E Slight	Wasted	Unilateral, multiple	Secondary cancer	L. 165 R. 500	++	Atrophy	None
(5) 461/51	M	71	Cardiac infarct	O E	Obese	Unilateral, multiple	Hypertensive atrophy	L. 210 R. 180	++	Slow necrosis	Patchy, complete
(6) 485/51	M	66	Glioblastoma multiforme	— —	Normal	Unilateral, multiple	Slight atrophy	L. 150 R. 120	++	Slow necrosis	Many, complete
(7) 55/52	F	73	Repeated cardiac infarction	O E	Obese	Bilateral, multiple	Hypertensive atrophy	L. 115 R. 100	++	Atrophy, slow necrosis	Some complete (severe in kidney)
(8) 197/52	F	61	Astrocytoma	— —	Normal	Unilateral, multiple	None	L. 185 R. 120	++	Atrophy, acute necrosis	Many, complete
(9) 400/52	F	68	Diabetes, cardiac infarction	— —	Obese	Bilateral, multiple	Atrophy from diabetic arteriolosclerosis	L. 175 R. 165	++	Normal	Few, complete
(10) 459/52	F	61	Reticulum cell sarcoma	— —	Moderate wasting	Bilateral, multiple	Acute inflammation, tumor invasion	L. 150 R. 225	++	Slow necrosis	Patchy, complete
(11) 11/53	M	80	Hematemesis, cirrhosis	— —	Moderate wasting	Unilateral, multiple	Senile atrophy	L. 120 R. 120	++	Atrophy	Few
(12) 18/53	F	44	Mitral stenosis, recurrent cerebral embolism	O E	Wasted	Bilateral, multiple	Congestion	L. 215 R. 215	+	Atrophy, slow necrosis	Few
(13) 34/53	F	63	Carcinoma of breast	— —	Wasted	Unilateral, multiple	Senile atrophy	L. 130 R. 130	+	Slow necrosis	Few
(14) 45/53	M	78	Carcinoma of colon	— E	Wasted	Bilateral, multiple	Atrophy, chronic inflammation	L. 180 R. 170	++	Slow necrosis	None

shell due to senility, atheroma, or hypertensive vascular disease. The weights of these kidneys show that this atrophy of the parenchyma was balanced by the excessive fat in the renal sinuses.

Differential Diagnosis

The only cysts likely to be confused with the ones that have been described are retention cysts of the renal tubules which have expanded into the fat of the sinus. These are of varying size and sometimes are multiple. On close inspection, their base of origin from the renal tissue can be discovered and the lining at that point usually is ridged where the cyst has expanded through the fibromuscular capsule. The wall is usually thicker and more opaque. In early hydronephrosis the fluid draining from the kidney into the lymphatics of the renal sinus may form pools there, but these pools have no limiting membrane and contain quantities of protein coagulated by the fixative.

Pyelo-sinusoidal backflow as a cause of these cysts is but a theoretic possibility. The only occasion on which I have ever observed communication between the renal sinus and a calyx was in a fulminating pyelonephritis in which the fornix was ulcerated. No cysts were present in the surrounding fat.

Hemorrhage into the fat of the renal sinus is very common but the blood is rapidly absorbed, usually without fibrosis, and hemorrhage is not a likely cause of these cysts.

Microscopic Appearance of Cysts and Surrounding Fat

The lining of the cysts consisted of a single layer of flattened mesenchymal cells which resembled the normal connective tissue boundary of a fat lobule. In one case the cysts were obviously of long standing and the surrounding fat was normal. In the remainder, changes were present which made it possible to follow the evolution of the cysts. These changes were (1) acute necrosis, (2) slow necrosis, (3) atrophy.

Acute Necrosis

Acute necrosis of the fat of the renal sinus was found only once (case 8), an indication that such a change is probably not a common precursor of the cysts. The lesion took the form of a small lemon-yellow area held in place in the darker surrounding adipose tissue by tenuous radiating bands which ran through a moat-like peripheral zone of oily fluid. This lesion was too soft and amorphous to be sectionable by the freezing microtome. In paraffin section the lesion contained no nuclei. In the central yellow area the outlines of the fat

cells were still visible but in the peripheral zone all traces of fat had disappeared and the only structures remaining were short, branching eosinophilic trabeculae which apparently were derived from condensation of the necrotic capillary network. Lines of mononuclear cells marked the margins of the lesion and were plentiful in the interstices of the surrounding fat. There was no evidence of acute inflammation. Arteriolosclerosis was widespread and an artery on the edge of the lesion was hyaline and had its lumen further reduced by branching intimal cells. This lesion differed from common focal necrosis of adipose tissue in that amorphous or crystalline products of lipolysis were absent. The fat liberated by necrosis of the fat cell was apparently being absorbed by mononuclear phagocytosis.

The fat of the renal sinus has an extremely good blood supply with many rich anastomoses, and at first thought it is hard to believe that acute necrosis of this type could have been due to vascular occlusion. Most of the fat, however, is composed of leaflets of adipose tissue arranged around a terminal arteriole. Capillary anastomoses may be scanty (Flemming[2]) and each leaflet depends largely on the integrity of its arteriole. It is therefore believed that this focal necrosis was ischemic in origin and that the area of acute necrosis was undergoing transformation into a cyst by slow absorption of the dead stroma and the liberated oil. The absence of fat-splitting activity was responsible for the absence of tissue reaction, and a delicate lining of the cyst would be formed by the natural mesenchymal boundaries of the lobules surrounding the necrotic one.

Slow Necrosis

Slow necrosis of the adipose tissue was found in 11 of my cases. It is recognized, of course, that where necrosis is concerned, the microscopic picture at any one moment is not a reliable guide to the rate of change, so the use of this heading is a matter of convenience. It covers the regressive changes which lie somewhere between simple atrophy and the acute focal lesions already described. Slow necrosis of a whole or a part of a lobule can be recognized when it is observed before absorption is complete. The skeletons of the cell boundaries are the last to be absorbed and lie attenuated, nucleus-free, and pale-staining. Lesser degrees of this process are not easy to recognize, for when the nuclei of one or two fat cells disappear their absence is not obvious. One is therefore dependent on finding cells whose nuclei are partially lysed. Two other features give a clue to the active loss of fat cells. The first is that when a fat cell dies its membrane ruptures and the site of damage is often marked by a cluster of phagocytes which

have taken up the liberated oil. The second is that in dying adipose tissue there often appear very long, narrow, pointed cells with elongated, dark nuclei. I have called these "splinter cells" and at first it was thought that they represented fat cells whose membrane had ruptured. It is now considered that they represent the free fibrocytes which may be found in the fatty lobule (Flemming[2]), and which are made prominent by loss of the fat cells. Their nuclear pattern differs from that of atrophying fat cells. The latter become rounded and pale staining. The "splinter cells" remain small and dark and eventually condense in parallel laminae along the outer borders of the pelvis or blood vessels. These "splinter cells" may also be seen in the renal sinus of the fetus before the fat cells have acquired fat.

In the causation of slow necrosis it is possible that ischemia also was a factor, since, in 2 cases there was severe, smudgy, hyaline obliteration of the arterioles and similar but patchy changes in 9 cases. The regressive changes had a predominantly lobular distribution, some lobules being reduced to vestiges of adipose tissue while neighboring ones were normal. The spaces so left were filled by fluid, and the boundaries of these developing cysts were again provided by the normal mesenchymal capsule of the neighboring lobules. The capillaries shared in the regressive changes. They were narrowed, reduced in number, and the normal network was unrecognizable.

Simple Atrophy

It has been stated already that in all of these kidneys excessive fat was present in the renal sinus and this was often associated with general obesity. Nevertheless, in 5 cases the margins of the adipose tissue in the renal sinus showed areas of simple atrophy in which the vascular network was condensed, interstitial fluid abundant, distended fat cells less numerous than might be expected, and the cytoplasm of the remaining fat cells swollen and contained a few small vacuoles.

Genesis of the Cysts

Arteriolosclerosis and regressive changes in fat are, of course, common findings in persons near the seventh decade. Some combination of circumstances must be sought to explain the severity of the changes and the formation of cysts around the renal calyces.

It is well recognized that the volume of fat in the renal sinus is often complementary to the amount of renal tissue. For example, in kidneys which have been hypertrophied for some time the amount of fat in the sinus is very small. Conversely, in slow atrophy of the kidney it is common for the external measurements of the kidney and the

total weight to remain fairly constant, the disappearance of parenchyma being compensated for by increase in the fat of the sinus. This latter condition was present in 9 cases in the present series.

The presence of a large volume of fat inside the partly enclosed cavity of the sinus creates a problem in adjustment when rapid reduction of the fat occurs. Such rapid reduction may occur because of a general demand made on body fat, as in rapid wasting or because of localized atrophy or necrosis caused by vascular disease. The key to the formation of the paracalyceal cysts lies in the question as to what is going to take the place of the fat, for the surrounding renal shell is not yielding enough to fall in and obliterate the cavity by pressure. A similar problem exists in the enclosed space of the marrow. The answer in both places is the same, transudation of fluid from the capillaries. In the marrow this leads to tiny cysts full of fluid. In the renal sinus it leads to cysts which may grow by confluence until they have replaced nearly all of the fat in the sinus. There is no reason why these cysts should produce any pressure on the calyces and it is therefore doubtful if they would modify the pattern of retrograde pyelography. The chief interest in them is as an illustration of problems of tissue balance in special sites.

Summary

Fourteen cases are described in which the fat beside the renal calyces contained thin-walled cysts.

The cysts occurred only in kidneys of which the fat in the renal sinus was abundant because of renal atrophy.

It is concluded that these cysts were due to fluid replacement of adipose tissue, which had undergone regressive changes owing to localized vascular disease and/or atrophy due to recent wasting.

Diminution of adipose tissue left a space which was prevented from collapsing by the surrounding shell of renal tissue and which therefore became filled with a fluid transudate.

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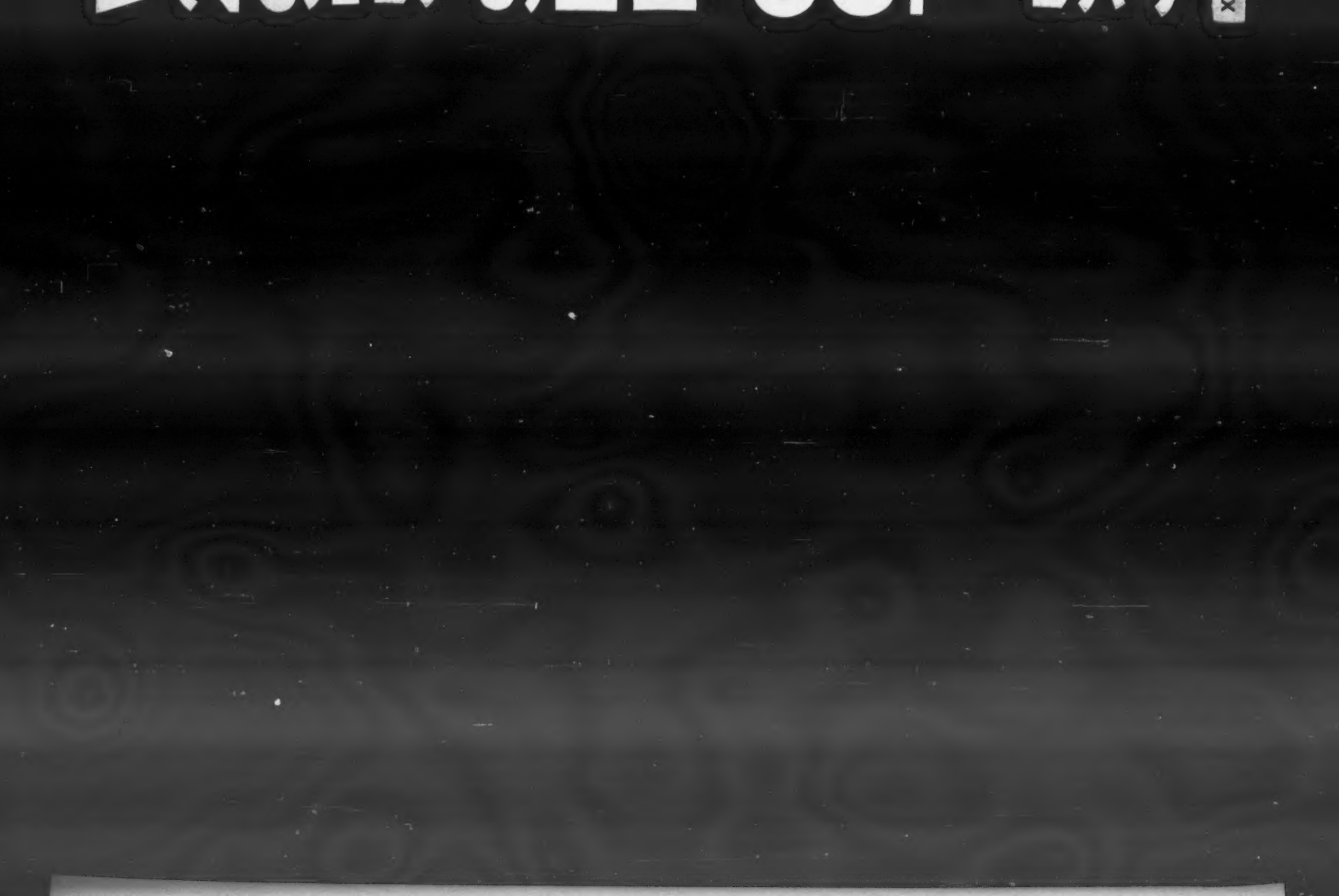
2. Flemming, W. Beiträge zur Anatomie und Physiologie des Bindegewebes. *Arch. f. mikr. Anat.*, 1876, **12**, 434–507.

LEGENDS FOR FIGURES

FIGS. 1 to 3. Case 14. Stages in the development of paracalyceal cysts. *Fig. 1.* Advanced cavitation of fat. *Figs. 2 and 3.* Units showing earlier changes.

FIG. 4. Case 1. A and B are calyces, both of which are surrounded by cysts formed by excavation of the fat in the sinus.



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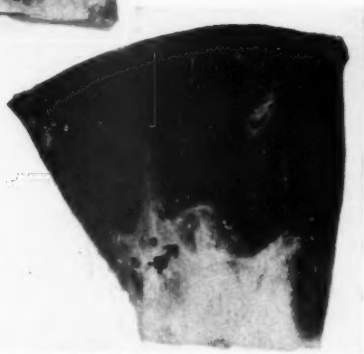

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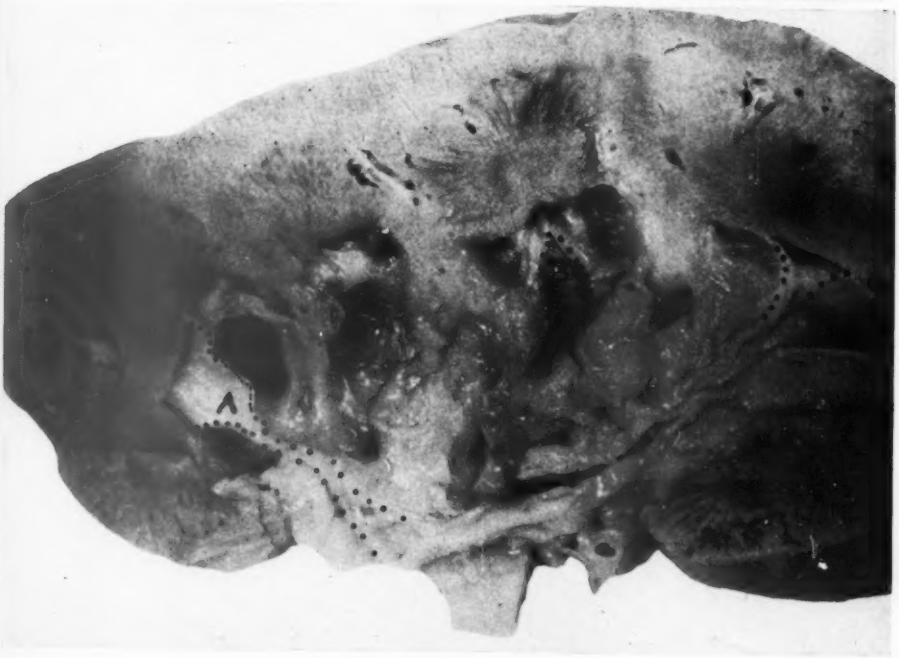

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